

Morphology and Development of the Postcranial Skeleton in the Channel Catfish *Ictalurus punctatus* (Ostariophysi: Siluriformes)

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Abstract

The morphology and development of the postcranial skeleton of three independent series of the channel catfish *Ictalurus punctatus* were studied using a combination of techniques (histology, SEM, skeletal clearing and staining). More than 2,000 specimens ranging in developmental stage from a fertilized egg to individuals about 400 mm TL were examined. Our results show individual variation in the onset of the hatching and foraging periods, and in the appearance or number of several skeletal structures (e.g., number of hypurals). Our results also show that regardless of this variation, the sequence of development of the postcranial structures is consistent within the series studied, and that the development of these structures is correlated more with the size of the fish than with age. Because of the consistent pattern of postcranial skeletal development observed, we were able to construct an ontogenetic staging scheme consisting of 18 developmental stages, each characterized by one defining criterion. Additional and more variable characters that occur concurrently with each of the 18 defining criteria are identified as concurrent features. This staging method facilitates future comparisons with the developmental patterns of other fish taxa, and is independent of age.

As part of this study, careful developmental descriptions of the Weberian apparatus, vertebral column, and paired and median fins were made. A primary goal of the study was to better understand the developmental relationship between the Weberian apparatus and the dorsal fin skeleton. Together they form an extremely unusual anatomical complex whose development and function are tightly linked. During development, the fourth neural spine of the Weberian apparatus forms a tight articulation with the first two proximal radials of the dorsal fin. In catfishes that exhibit a similar modification of the dorsal fin, sound production has been implicated.

Through detailed anatomical descriptions, this study examined contested homologies of the vertebral column and caudal fin. Such homologies include the caudal fin epurals, which in *Ictalurus punctatus* form as independent elements that later fuse to the neural spines of posterior vertebrae. New terminology is suggested for several skeletal structures to reflect their developmental origin.

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Introduction

Until recently, researchers examining patterns of vertebrate development, and more specifically actinopterygian development, focused on a handful of "model species" (e.g., the zebrafish *Danio rerio*) to make generalizations about the formation of morphological structures such as the neural tube and vertebral column (Kuwada et al., 1990; Kimmel et al., 1995). However, studies such as those of Schultze and Arratia (1988, 1989), Bemis and Grande (1992), Arratia and Schultze (1992), Shardo (1995), and Grande and Bemis (1998) have illustrated significant variation in skeletal development within Actinopterygii. Obviously, no one fish species can be used as a model for all of Actinopterygii.

This study describes the development of the postcranial skeleton (post early cleavage stages) of *Ictalurus punctatus*, commonly known as the channel catfish, one of the most common ictalurids in North America. Phylogenetically, *I. punctatus* is considered by many researchers (e.g., Lundberg & Baskin, 1969; Arratia, 1992; de Pinna, 1996; Coburn & Grubich, 1998) to be a relatively basal siluriform because it exhibits a primitive caudal fin and a relatively primitive Weberian apparatus in comparison to other catfishes. On the other hand, as a siluriform, *I. punctatus* exhibits many modifications and bone fusions not present in outgroup ostariophysans such as cypriniforms, characiforms, and goniorhynchiforms (Rosen & Greenwood, 1970; Fink & Fink, 1981, 1996; Grande & Poyato-Ariza, 1999).

Although specimens of adult catfishes are common in North American museum collections, developmental material and consequentially developmental studies are rare (e.g., Armstrong, 1962; Arratia, 1990, 1992; Kobayakawa, 1992; Adriaens, 1998; Coburn & Grubich, 1998). Studies of the development of *I. punctatus* are rarer still, and have focused only on specific parts of the skeleton. For example, Kindred (1919) and Eaton (1937) examined skull development, while Al-Rawi (1966) examined the early development of the Weberian apparatus. The current study examined the development of the entire postcranial skeleton (vertebral column, Weberian apparatus, dorsal and caudal fins, and pectoral and pelvic fins) using three independent developmental series, each from a single spawn, for a total of more than 2,000 specimens. We found that, contrary to Lundberg and Baskin (1969), the caudal fin

skeleton of *I. punctatus* exhibits considerable morphological variation within and between the developmental series examined. Despite this variation, consistent features of axial skeleton formation can also be identified, and the detailed developmental timing of particular skeletal elements appears to correlate more closely with the size of the fish than with its age.

Hypotheses about the evolution, development, function, and homologies of different elements of the Weberian apparatus have been proposed by many authors (e.g., Krumholz, 1943; Martin, 1963; Alexander, 1964; Rosen & Greenwood, 1970; Gayet, 1986; Chardon & Vandewalle, 1997). Some of these hypotheses are in conflict with each other. For example, Coburn and Futey (1996) argue that the claustrum in otophysans is derived from the first supraneural, while Fink and Fink (1981) argue that the claustrum forms from a disassociated part of the first neural arch. As part of our study we investigated various hypotheses of Weberian apparatus formation in light of the new morphological data we obtained. Our observations show that the development of the Weberian apparatus in *I. punctatus* is functionally linked to the development of the dorsal fin skeleton. We term this functional unit the Weberian apparatus/dorsal fin unit, and explore some implications of this unit as a stabilizer for the dorsal fin spine. We also comment on its possible role in sound transmission.

Methods

Skeletal Preparation

Specimens were preserved in either 10% buffered formalin or 4% buffered paraformaldehyde. Skeletal material was prepared using a modified version of Dingerkus and Uhler's (1977) technique for staining and counterstaining bone and cartilage. In this method, bone is stained with alizarin red and cartilage is stained with alcian blue. Trypsin was used to render the soft tissue transparent. An ethyl alcohol series was substituted for the KOH step in the standard Dingerkus and Uhler method because the larval specimens are fragile. Once cleared and stained, the specimens were stored in glycerin. Specimens were dissected, examined, and drawn under a Wild MZ8 dissecting microscope. Total lengths (TL) were taken from all specimens. Standard lengths (SL) were

recorded for specimens with defined caudal fin supports.

Histological and SEM Preparation

Formalin-fixed specimens ranging in size from 5 mm to 20 mm TL were prepared for histological examination using one of two methods. To help establish the onset of vertebral column ossification, specimens were decalcified in De-cal (National Laboratories), dehydrated in an ethyl alcohol series, cleared in xylene, embedded in paraffin, and cut in 10- μ m-thick transverse or sagittal sections. To examine the morphology of the Weberian apparatus and vertebral column, specimens were prepared using the low-viscosity nitrocellulose (LVN) embedding technique of Thomas (1983). Specimens were decalcified in formic acid, dehydrated in an alcohol series, and embedded in a graded concentration series of LVN. Transverse sections 40 μ m thick were cut using an American Optical 860 sliding microtome. Both thin and thick histological sections were stained with a modified version of Humason's (1972) hematoxylin and picro-ponceau procedure.

Embryonic (i.e., prehatch) and yolk sac larval specimens were studied with scanning electron microscopy (SEM). For embryonic specimens, the chorion was removed as the first step. Dechorionated embryos and yolk sac larvae were washed in sodium cacodylate buffer and then postfixed in an aqueous solution of 2% osmium tetroxide. Each specimen was then dehydrated through an alcohol series, treated with Peldri II, dried in air, coated with 400 A of gold/palladium (Young et al., 1995), and examined in a Cambridge 240 SEM.

Developmental Staging Method

Early ontogeny consists of growth and a sequence of developmental changes over time. These rates of growth and developmental change are not necessarily constant or correlated with each other. Thus, age or length does not consistently correspond to a level of development, particularly among different species (Fowler, 1970; Reimchen & Nelson, 1987). For purposes of comparison, in this study the developmental sequence of the axial skeleton is divided into morphological stages. Each stage is characterized by one morphological feature that acts as the defining criterion (Shardo, 1995). Additional and more variable

characters that occur concurrently with the defining criterion of a particular stage but are not necessarily linked to the defining character are defined as concurrent features (Shardo, 1995). This method of developmental staging allows for comparisons with other species, regardless of the length or age of the individuals being compared.

Materials

Developmental Series Examined

Three independent developmental series of channel catfish were examined. Two separate series were spawned from eggs obtained from Osage Catfisheries (Osage Beach, Mo.). The third series was spawned from eggs obtained from the Catfish Genetics Research Unit (U.S. Department of Agriculture, Agriculture Research Services, Stoneville, Miss.). Each developmental series was raised at a different location but under approximately the same conditions (i.e., simulated stream facilities at about 26°C). Series A was raised by Grande in a greenhouse facility with natural lighting at Loyola University, Chicago. Series B was raised by Bemis laboratory personnel working at the Osage Catfish facility and obtained from W. E. Bemis (University of Massachusetts, Amherst). Series C was raised by Shardo at the Stoneville Catfish Facility. A total of 2,293 specimens (985 specimens in series A, 258 specimens in series B, 1,050 specimens in series C) were collected from these series, ranging from newly fertilized eggs to foraging subadults with ossified axial skeletons. Samples of at least seven specimens were collected for 18 days (series B) and 30 days (series A and C). At least four samples were preserved each day within the collecting period. The number of samples and the frequency of collecting decreased in all three series toward the last days of collecting, after the fish had achieved a total length of 35–40 mm or were foraging freely.

All fish from series A are deposited in the fish collection at Loyola University, Chicago (LU D081090). Illustrated specimens were assigned Loyola University (LU) catalogue numbers. Series B is deposited in the University of Massachusetts Ichthyological Collection, Amherst (UAM F11257), and series C is housed at the University of South Alabama, Mobile (USA 040645).

Adult Materials Examined

Materials examined to assess the morphological condition of the postcranial skeleton in larger specimens and related taxa are as follows:

- Clarias liberiensis*: 1 specimen (SL = 60 mm): FMNH 50272 (cleared and stained).
Corydoras aeneus: 3 specimens (SL = 42–69 mm): FMNH 54832 (cleared and stained).
Doras carinatus: 1 specimen (SL = 110 mm): FMNH 53192 (cleared and stained).
Helogenes marmoratus: 1 specimen (SL = 60 mm): FMNH 70115 (cleared and stained).
Ictalurus punctatus: 9 specimens (SL = 160–400 mm): FMNH 16711, 86267, 6430, 63699 (skeletons); LU F082256, F082257, F082258, F082259, F082260 (cleared and stained, alcohol).
Ictalurus balsamus: 2 specimens: FMNH 51269 (disarticulated skeletons).
Ictalurus furcatus: 1 specimen: FMNH 73900 (very large disarticulated skeleton).
Noturus gyrinus: 1 specimen: (SL = 56 mm): FMNH 42269 (cleared and stained).
Rhamdia nicaraguensis: 1 specimen: (SL = 150 mm): FMNH 5904 (cleared and stained).
Rhamdia nasuta: 1 specimen: (SL = 75 mm): FMNH 35319 (cleared and stained).
Trachelyopterichthys taeniatus: 1 specimen: (SL = 65 mm): FMNH 69929 (cleared and stained).
Trichomycterus banneai: 2 specimens: (SL = 65–71 mm): FMNH 70014 (cleared and stained).
Trichomycterus laticeps: 2 specimens: (SL = 38–45 mm): FMNH 79128 (cleared and stained).

Abbreviations

The following abbreviations are used for institutions and anatomical terminology:

- FMNH Field Museum of Natural History, Chicago
LU Loyola University, Chicago
UAM University of Massachusetts, Amherst
- act actinotrichia
anp anterior nuchal plate
ar anterior radial (= supraneural of Grande & Lundberg, 1988; pterygiophore of Fink & Fink, 1996)
boc basioccipital
br bony ridge
chc chordacentrum (*sensu* Schultz & Arratia, 1986)
cl claustrum
darc dorsal arcocentrum (*sensu* Schultz & Arratia, 1986; = basidorsal of Patter-

- son, 1968; Schultz & Arratia, 1986; Grande & Bemis, 1998)
- df dorsal fin
df1 dorsal fin spine 1
df2 dorsal fin spine 2
dl dorsal lamina
dr1–7 dorsal radials 1–7
ep epural
ha hemal arch
hyl–6 hypural 1–6
in intercalarium
lpdt lepidotrichia
na neural arch
nc notochord
ns neural spine
ns4 neural spine 4
nt neural tube
os os suspensorium
pf pectoral fin bud
phy parhypural
pnp posterior nuchal plate
pr proximal radial
pu preural centrum
p4a anterior part of the transverse process
p4p posterior part of the transverse process
rn radial nodule
sc scaphium
sn supraneural
soc supraoccipital
tp4 transverse process 4
tr tripus
u1–2 ural centrum 1–2
un uroneural
v1–10 vertebra 1–10
varc ventral arcocentrum (*sensu* Schultz & Arratia, 1986; = basiventral of Patterson, 1968; Schultz & Arratia, 1986; Grande & Bemis, 1998)

Results

Although some individual variation between and within particular series of *I. punctatus* is evident (such as in the caudal fin skeleton), the basic pattern and sequence of bone ossification is consistent among the three series. As modified from Bemis and Grande (1992) and Shardo (1995), we divide the development of *I. punctatus* into three major periods: the embryonic period (defined as the period from fertilization to hatching), the yolk sac larval period, and the foraging period.

The onset of the particular developmental pe-

TABLE 1. Summary of samples exhibiting first occurrence of defining criteria.

Stage	Series A (LU D.081090)	Series B (UAM F11257)	Series C (USA 040645)
1	(Day 2, sample 7)	(Day 2, sample 8)	(Days ?–5, samples ?–40)
2	7.6 mm (day 3, sample 10)	7.6 mm (day 4, sample 14)	8.2–9.5 mm (days 6–7, samples 41–50, hatching)
3	8.1–10 mm (day 4, sample 15)	8.6 mm (day 4, sample 17)	8.4–10.2 mm (day 7, sample 51)
4	9.4–10.7 mm (day 6, sample 20)	9.0 mm (day 5, sample 18, hatching)	9.9–12.2 mm (days 8–10, samples 52–55)
5	9.8–11 mm (day 6, sample 24, hatching)	9.7–10.0 mm (day 5, sample 20)	11.9–12.6 mm (day 11, sample 56)
6	10.2–11.5 mm (day 7, sample 28)	10.5–11.0 mm (day 6, samples 22–23)	11.5–13.6 (days 11–12, samples 56–57)
7	9.7–12.1 mm (day 8, samples 31–35)	10.9–12.2 mm (day 7, samples 24–26)	11.5–13.6 mm (day 12, sample 57)
8	12.0–13.6 mm (day 9, sample 39)	12.5–13.5 mm (days 8–9, samples 28–29)	11.5–13.6 mm (day 12, sample 57)
9	12.6–14.4 mm (day 10, sample 44)	14.2–14.3 mm (day 10, sample 32)	13.9–14.4 mm (day 13, sample 58)
10	14.4 mm (day 10, sample 46)	14.0–14.4 mm (day 11, sample 33)	13.4–16.1 mm (days 14–15, samples 59–60)
11	15.0 mm (day 11, sample 51)	14.8–15.0 mm (day 12, sample 34)	16.2–17.6 mm (day 17, sample 62)
12	15.0–15.5 mm (day 13, sample 53, foraging)	15.6–16.0 mm (day 13, sample 35, foraging)	16.9 mm (day 17, sample 62)
13	15.5–16.1 mm (day 14, sample 60)	16.5–17.5 mm (day 15, sample 36)	27.1–31.2 mm (day 36, sample 81, foraging)
14	16.9–18.1 mm (day 18, sample 78)	17.5–18.3 mm (day 17, sample 37)	27.1–31.2 mm (day 36, sample 81)
15	23.0–26.0 mm (day 27, sample 89)	21.7–24.0 mm (day 23, sample 39)	No samples
16	40.0 mm (day ?, sample 93)	30.5–32.9 mm (day ?, sample 40)	No samples
17	45.0–50.0 mm (day ?, sample 95)	No samples	No samples
18	50.0–60 mm (day ?, sample 95)	No samples	No samples

riods varied among the three series. This is unsurprising, because variations in the timing of the three developmental periods (e.g., number of days to hatching) could reflect the slightly different water temperatures or light regimens in the environments in which the fish were raised (Fowler, 1970). The sampling time periods for each catfish series are listed below and in Table 1. More important, our results show that the development of particular structures in *I. punctatus* is closely correlated with the length of the fish. This correlation is consistent with the findings of Faustino and Power (1998) for *Sparus aurata*.

The development of the axial skeleton is further divided into 18 stages (Table 2). The defining criteria proposed here are characters that show little or no variation in developmental sequence and are common to teleosts (e.g., the presence of hypural 1) or more specifically to ostariophysans. Defining criteria were easily observed in all three devel-

opmental series. The concurrent features listed in Table 2 include both postcranial and cranial characters that first appear in that stage in at least one of the three series. Any variation in the timing of the concurrent features among the series is mentioned in the description of each stage. Age and length of the specimens in a single stage varied somewhat among the three series and are treated as concurrent features.

Developmental Staging

Embryonic Period. The embryonic period for *I. punctatus* extends from fertilization to hatching. In the three series examined, this developmental period lasted about five to seven days. We cannot more precisely determine the length of the embryonic period for series A and B because the

TABLE 2. Defining and concurrent criteria for stages 1–18.

Stage	Defining criteria	Concurrent characters
1	Presence of 4 visceral arches; at least 50 somites formed	Notochord present but unconstricted; pectoral fin buds present; brain with three distinct divisions; presence of 1 continuous fin fold
2	Presence of hypural 1 of caudal fin	Parhypural and hypural 2 often but not always formed; actinotrichia formed in caudal fin; basidorsal forming; mandible, hyomandibula, otic capsule, and cranial floor forming in cartilage; utricular and saccular otoliths formed; opercular facet ossified; notochord constricted anteriorly
3	Presence of at least 4 cartilaginous hypurals	Basidorsals and basiventrals formed; orbital and epiphyseal bar forming; pterygoids and visceral arches 1–5 formed in cartilage; supracleithra and cleithra formed; pelvic fin buds present; dentaries begin to ossify in series A and B
4	Protocentra mineralize in anterior region of vertebral column	Formation of autogenous epural in caudal fin skeleton; formation of anal radials begins; presence of at least 7 cartilaginous proximal radials in dorsal fin; dentaries ossify in series C
5	Presence of ural centrum 1 ($\mu 1$)	Uroneural 1 ($un1$) formed in series C; parasphenoid begins to ossify; teeth on dentary, maxilla forming; presence of lateral line on dorsal skull roof marking the beginning of frontal bone formation in series A and B; basioccipital and exoccipitals ossifying; early ossification of hyomandibular in series C
6	Formation of ural centrum 2 ($u2$)	First appearance of Weberian apparatus ossicles (i.e., tripus, scaphium, intercalarium) and transverse process of vertebra 4; presence of all proximal dorsal fin radials; initial ossification of parhypural and hypurals in series C; formation of sixth hypural in large specimens
7	Neural arches meet and begin to ossify at dorsal midline posterior to dorsal fin	Ossification of Weberian ossicles begins; formation of teeth on epibranchial 4; presence of a supraneural anterior to the dorsal fin; formation of teeth on epibranchial 4; formation of first dorsal fin spine; formation of distal radials in anal fin in series C
8	Mineralization of all vertebral centra	Uroneural 1 elongates to the distal margin of $u2$; second dorsal fin spine forms; lateral line forms around orbit and preopercular region; ossification of parhypural in series A and B
9	Fusion of vertebral centra 2 and 3	Ossification of all branchiostegal rays; formation of distal radials of anal fin in series A and B; premaxilla with 1 row of teeth
10	First 2 dorsal fin radials enlarge and articulate with spine of neural arch 4 of Weberian complex	First appearance of a cartilaginous claustrum; formation of “epurals” associated with posterior 7 preural centra; tooth formation on both ventral and dorsal gill arches continues; presence of 5 distal radials of dorsal fin; formation of basal segment of pectoral fin spine
11	Fusion of vertebrae 2, 3, and 4	Elongation of “epurals”; formation of the basal segment of pectoral fin spine in series A and B; ossification of pectoral girdle and fins in series C
12	Transverse process of vertebra 4 of Weberian apparatus articulates with pectoral girdle	Hyomandibula begins to ossify in series A and B; neural and hemal arches are ossified
13	All Weberian ossicles are ossified	Each half of the neural arch of vertebra 5 meets along dorsal midline
14	All hypurals are at least two-thirds ossified	All “epurals” are at least on-half ossified; most skull bones (e.g., supraoccipital, pterygoids) have formed and are well-ossified; caudal fin forks and looks like adult structure

TABLE 2. *Continued.*

Stage	Defining criteria	Concurrent characters
15	Proximal radials of anal fin ossify	Pectoral girdle ossified in series A and B; all but last dorsal fin radial are ossified
16	Completion of Weberian/dorsal fin complex	Fusion of "epurals" with corresponding neural spines in series A and B
17	Hypurals 3 and 4 form a unit with u2	Completion of skull lateral line system; continued ossification of skull and growth
18	Fusion of pu1, u1, un1, and hypurals 1 and 2, forming compound centrum of caudal fin	Fusion of hemal spines to corresponding centra, anterior to pu2; adult coloration observed

Note: Defining criteria and concurrent characters are explained in the text.

precise time of fertilization was not recorded, although it is known that fertilization occurred about one day prior to the first collection. A more precise fertilization time is known for series C because the eggs were fertilized by sperm in the laboratory.

Series A: July 9–July 13/14 (stages 1–5, day 6)

Series B: May 29–June 3 (stages 1–4, day 5)

Series C: June 25–June 30 (stages 1–2, day 6–7)

Yolk Sac Larval Period. This period extends from hatching to the complete depletion of the yolk sac. During this time all postcranial axial skeletal elements are formed, although not completely ossified (e.g., caudal fin hypurals). The starting date for the yolk sac larval period was determined when the majority of the fishes in each series had hatched.

Series A: July 15–July 19 (stages 6–11, ending on day 11)

Series B: June 4–June 11 (stages 5–11, ending on day 12)

Series C: June 30–July 15 (stages 3–12, ending on day 17)

Foraging Period. The start of the foraging period is characterized by the disappearance of the yolk sac. The fishes are now foraging on their own. It is a time of continued skeletal ossification and growth. Foraging was determined either by observing food in the gut tract of a fish or by direct observation of a fish feeding.

Series A: July 20–Sept. 15 (collections terminated) (stages 12–18)

Series B: June 12–June 22 (collections terminated) (stages 12–16)

Series C: July 16–July 30 (collections terminated) (stages 13–14)

Stage 1

This study is concerned with the development and ossification of the postcranial skeleton, not with early cleavage stages. We thus begin by describing the level of development of specimens just prior to the formation of elements in the postcranial skeleton. These specimens already exhibit head lift (Fig. 1A). At this stage the optic vesicles are formed, the division of the brain into three primary brain regions (prosencephalon, mesencephalon, and rhombencephalon) is obvious, the branchiomeres have undercut the head laterally, four visceral arches have formed, at least 50 somites are present, Meckel's cartilage and the maxillary barbels are beginning to form, and the pectoral fin buds first appear. Also in stage 1, the fishes exhibit an unstricted notochord and a continuous fin fold. Neural and hemal arches, hypurals, and median fin pterygiophores are not yet present.

Stage 2

This stage is characterized by the presence of a cartilaginous hypural 1 in the caudal fin skeleton. In most specimens examined the parhypural and hypural 2 are also present in cartilage. The appearance of hypural 1 is quickly followed by the formation of additional hypurals. We found only a few specimens of stage 2 in which hypural 1 was the only hypural in the caudal region. Actinotrichia are present in the ventral part of the caudal fin only. The notochord shows a series of constrictions resulting in a series of protocentra (i.e., centra precursors; Arratia, 1991; Grande & Bemis, 1998). In the skull, Meckel's cartilage plus the cartilaginous hy-

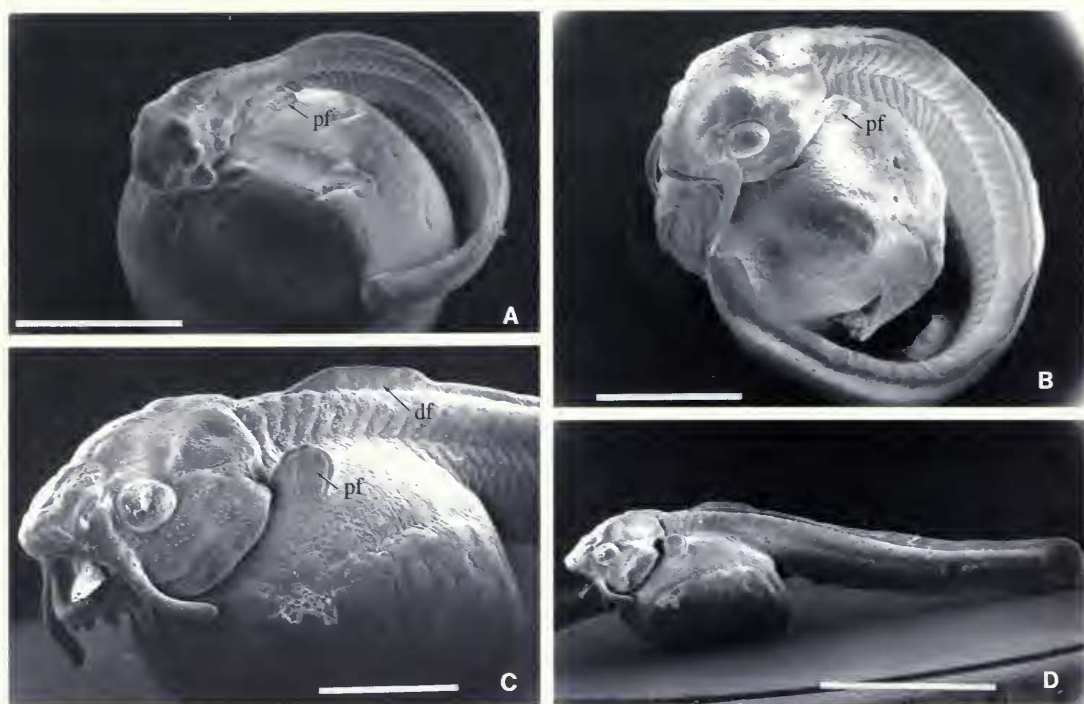


FIG. 1. SEM images of early developmental stages of *Ictalurus punctatus*. **A**, Stage 1, showing the early development of the pectoral fin buds, head lift, and the early formation of the maxillary barbel. Scale bar = 1 mm. **B**, Stage 3. Scale bar = 1 mm. **C** and **D**, Stage 4, showing the development of the pectoral fin buds, the dentary barbels, and the early formation of the dorsal fin. Scale bar = 2 mm in C, 1 mm in D. For abbreviations, see p. 4.

omandibula and the otic capsule are forming. The utricular and saccular otoliths have formed, and skull dermal bone formation begins with the opercular facet of the opercular bone (i.e., the articular surface of the opercle for the articulation with the hyomandibula).

Stage 3

At least four cartilaginous hypurals are formed at this stage (Fig. 3A). In addition, cartilaginous dorsal arcocentra are formed along the length of the constricted notochord and cartilaginous ventral arcocentra are found only posterior to the yolk sac. As discussed in Arratia and Schultze (1992), arcocentra will bear neural and hemal arches, respectively, and correspond to the basidorsals and basiventrals, respectively, of Schultze and Arratia (1986) and Grande and Bemis (1998). They thus form the chondral components of the vertebrae.

Other internal characters observable at this stage include the presence of a cartilaginous hyomandibula, pterygoids, and ceratobranchials 1–

5, as well as a cartilaginous orbital and epiphyseal bar and lateral expansion of Meckel's cartilage. Ossification of the dentary was observed in stage 3 of series A and B but was delayed to stage 4 in series C. Ossification of the maxillae at the base of the maxillary barbel, supracleithra and cleithra, and enlargement of the opercle are also evident. Both the utricular and saccular otoliths are clearly visible in specimens at this stage.

Externally, the body and tail are still coiled around the yolk (Fig. 1B). An additional set of barbels has formed median to the maxillary barbels, which have elongated. The pelvic fin buds appear as small protrusions immediately posterior to the yolk sac. The pectoral fins have enlarged slightly with the formation of the basal scapulocoracoid cartilage. Distal to the scapulocoracoid cartilage is a sheet of condensing cartilage that will form the radials of the pectoral fin. The head is primarily free from the yolk, attached only in the region of the opercle, thus freeing the lower jaw and allowing the mouth to open.

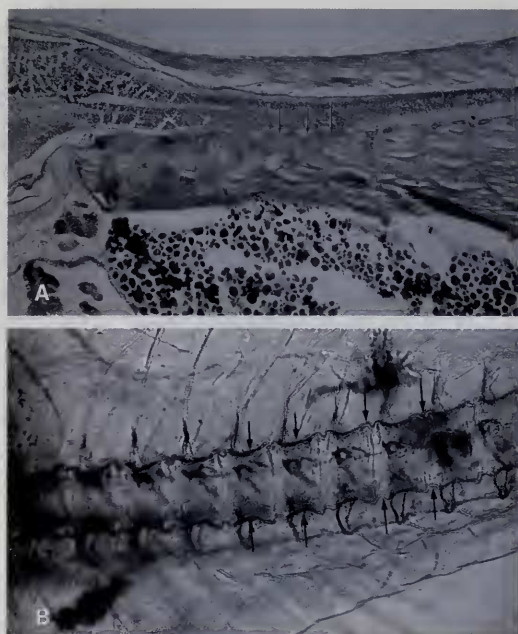


FIG. 2. **A**, Sagittal histological sections showing the first sign of protocentra mineralization. Arrows point to individual protocentra in a specimen 9 mm TL (stage 3–4). Anterior is to the left. **B**, Photograph of cleared and stained specimen (13 mm TL, LU F082289, stage 8) showing mineralization of the chordacenta from within the notochordal sheath. Arrows point to the outer margin of the notochordal sheath. Anterior is to the left.

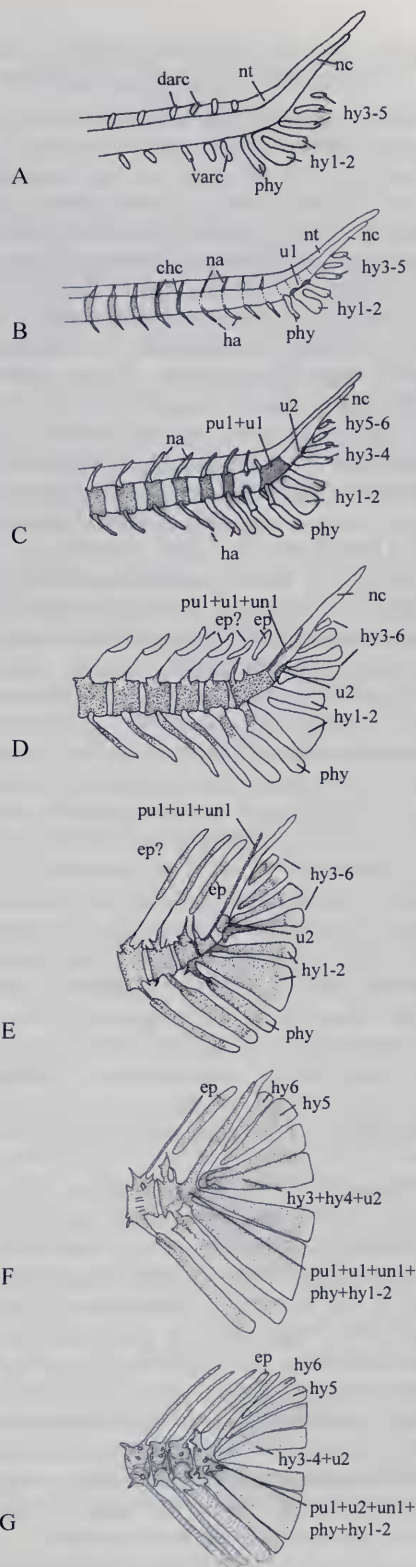


FIG. 3. Development of the caudal fin skeleton from an unconstricted notochord stage to the formation of the compound terminal centrum. **A**, Embryo, 9.2 mm TL (stage 3) (LU F082275). **B**, Embryo, 10.4 mm TL (stage 5), showing the formation of ural centrum 1 (u1). Note that u1 begins to form before the posterior preural centra (LU F082276). **C**, Yolk sac larva, 11.5 mm TL (stage 6), showing the formation of ural centrum 2 (LU F082277). **D**, Yolk sac larva, 13.1 mm TL (stage 8), showing the formation of the compound centrum, the autogenous epural, the median “epurals,” and ossification of the parhypural. Only the first five median “epurals” are illustrated (LU F082278). **E**, Foraging juvenile, 20 mm TL (stage 14–15), showing further development of caudal skeleton (LU F082279). **F**, Foraging juvenile, 40 mm TL (late stage 15), showing the association of hypurals 3 and 4 with u2 (LU F082280). **G**, Subadult, 168 mm TL (no stage), showing a fully formed caudal fin skeleton (LU F082281). Anterior is to the left. Ossification of hypurals and mineralization of centra are shown with stippling. Unstippled areas are cartilaginous. For abbreviations, see p. 4.

Stage 4

Stage 4 marks the beginning of vertebral centra formation. Fishes at this stage in series A and B are 5–6 days old and have not yet hatched, but they are 8–10 days old and post-hatching in series C. After the notochord has constricted and the arcocentra have formed, chordacentra (“ring centra,” Lundberg & Baskin, 1969; Collette et al., 1983; Schultze & Arratia, 1989; Arratia, 1991) begin to develop from within the notochordal sheath of each protocentrum (Laerm, 1982; terminology of Grande & Bemis, 1998). In *I. punctatus*, mineralization of dorsal and ventral chordacentra occurs simultaneously. Each dorsal and ventral pair of chordacentra quickly enlarges, fuses, and forms a ring around each protocentrum (Figs. 2A and B). Although at this stage in development only a thin mineralized surface layer is present on each protocentrum (most protocentra have mineralized in specimens of 10–11 mm TL), the chordacentra will quickly thicken, and autocentra, which arise as direct ossifications, will surround each chordacentrum. The autocentra (observed in individuals about 13 mm TL; see Stage 6) will in turn enlarge and complete the formation of the vertebral centra. Chordacentrum formation in *I. punctatus* is unlike that reported by Arratia and Schultze (1992) for *Oncorhynchus* and several other salmonids, which primarily exhibit only ventral chordacentra. In salmonids, chordacentra enlarge dorsally and are eventually supplanted by autocentra. Not surprisingly, variation in chordacentrum formation is present among teleosts, but the dorsal–ventral pattern observed in *I. punctatus* seems fixed among ostariophysans. Chordacentrum formation in *I. punctatus* is accompanied by a straightening of the trunk and tail (Figs. 1C and D).

Concurrent features present at this stage include an autogenous cartilaginous epural in the caudal fin skeleton, lepidotrichia in the entire caudal lobe, a minimum of seven cartilaginous proximal radials in the dorsal fin, rudimentary ventral arcocentra above the yolk sac, and a third pair of barbels under the chin. In the pectoral fins the scapulocoracoid cartilage is enlarging and three proximal radials are present. The medialmost pectoral radial appears continuous with the scapulocoracoid cartilage. With the exception of the anterior radial in the dorsal fin, radials in all fins form and ossify from rostral to caudal.

During this time the cartilaginous proximal anal fin radials form. In the specimens examined the

proximal anal fin radials begin to form in an anterior to posterior direction before the anal fin lobe forms. In siluriforms the middle radials of both the anal and dorsal fins are absent as separate elements. It has been suggested that the middle radials are lost (Fink & Fink, 1981) or fuse with the proximal radials (Grande, 1987). In the specimens observed, the proximal radials formed as single and solid forms. Slightly later in development, the distal radials form between the right and left halves of the lepidotrichial bases.

Stage 5

Stage 5 is defined by the presence of ural centrum 1 (u1). As observed in *Amia calva* (Grande & Bemis, 1998) and *Oncorhynchus mykiss* (Arratia & Schultze, 1992), ural centrum 1 in *I. punctatus* begins to mineralize before mineralization of the abdominal protocentra is complete (Figs. 3B and C). Ural centrum 1, however, mineralizes differently from the other centra. Instead of the simultaneous dorsal–ventral pattern of the abdominal centra, ural centrum 1 mineralizes from the ventral side of the protocentrum. Growth of ventral chordacentra forming the ural centra appears to be common among primitive teleosts (Schultze & Arratia, 1988, 1989). Ural centrum 1 throughout its development is associated with hypurals 1 and 2 only. An unmineralized protocentrum lies dorsal to the parhypural at this stage and is presumed to be the precursor to preural centrum 1 (pu1).

Concurrent characters include the complete mineralization around the anteriormost protocentra in series A and B, but around over two-thirds of the total protocentra (beginning anteriorly) in series C. Also in series C, uroneural 1 (un1) was observed extending from the completed postero-dorsal corner of ural centrum 1. This single uroneural eventually extends along the dorsolateral margin of the notochord. It thickens and straightens into a rodlike structure and, together with the epural, stiffens the epaxial portion of the caudal fin (Lauder, 1989). The early formation of uroneurals (i.e., membrane versus cartilage bone) varies among teleosts (Greenwood, 1966; Patterson, 1968; Schultze & Arratia, 1989; Arratia & Schultze, 1992). Our observations show that uroneural 1 in *I. punctatus* forms as membrane bone without a cartilaginous precursor (Fig. 3D). This pattern of formation of uroneural 1 in *I. punctatus*

may be a synapomorphy of ictalurids and possibly siluriforms.

In the pectoral fins, the scapulocoracoid cartilage has elongated both dorsally and anteroventrally along the medial surface of the cleithra. Formation of pectoral fin rays has begun. In the more slowly developing pelvic fins, six fin rays extend out from a small cartilaginous bar in the pelvic fin buds. The parasphenoid begins to ossify, and by now at least one-half of the dentary is ossified and supports a single row of teeth. Four branchiostegal rays have ossified on each side of the skull, and the opercle continues to grow and ossify. In series A and B, lateral line canals were observed on the dorsal skull roof, marking the start of frontal bone formation. Fully formed frontal bones appeared in stage 6 in series C. The basioccipital and exoccipitals ossify at the anterior end of the notochord.

Stage 6

This stage is characterized by the mineralization of ural centrum 2 (u2). Ural centrum 2 forms from a ventral chordacentrum, as does ural centrum 1, and is closely associated with cartilaginous hypurals 3 and 4. In a few specimens of series C, cartilaginous hypurals 4 and 5 articulate with ural centrum 2. In most specimens examined ural centrum 2 never fuses with ural centrum 1.

Concurrently in series C (11.5–13.6 mm TL), ossification of the central portions of the parhypural and hypurals 1–5 begins. Ossification of the parhypural in series A and B does not occur until later in development (about stage 8), at around 13.5 mm TL. Hypurals in series A and B do not begin ossifying until after the parhypural begins to ossify. Larger specimens in all series often contain a sixth cartilaginous hypural. From this stage onward, a gradually increasing percentage of specimens examined contain a sixth hypural, which is very slow to ossify. In many specimens examined, specifically in series A and B, hypural 6 never develops. At this stage (10.7–13.6 mm TL) in all series, all dorsal fin radials are present, as well as at least six dorsal fin rays. In general, the skull bones exhibit slightly more ossification than in stage 5, but more important, this stage marks the first appearance of Weberian apparatus ossicles (i.e., tripus, scaphium, and intercalarium) and the transverse processes of vertebra 4. These elements, although preformed in cartilage as basidorsals and basiventrals, respectively, ossify very

rapidly, suggesting the importance of a functioning Weberian apparatus in larval catfishes.

Stage 7

Neural arches posterior to the dorsal fin meet along the dorsal midline and begin to ossify proximally. The formation of a neural arch bridge is typical for ostariophysans (Fink & Fink, 1981). Neural arches 5–11, which lie ventral to the dorsal fin radials, do not meet along the dorsal midline to form neural spines as do the more posterior arches. Instead they are separated and prevented from meeting by the elongation and intervention of the dorsal fin radials as the Weberian apparatus/dorsal fin complex forms.

The Weberian ossicles begin to ossify at this stage in fishes about 11 mm TL, starting with the tripus. Not surprisingly, the Weberian ossicles ossify before ossification of the more posterior neural or hemal arches is complete. During this stage hemal arches also meet in the midline, forming hemal spines posterior to the anus. Additional concurrent characters include the presence of teeth on epibranchial 4 of the gill arches, and formation of the first dorsal fin spine. The first spine will become the locking mechanism for the second fin spine in the fully formed dorsal fin. In addition, a supraneural forms anterior to neural arch 3 of the Weberian apparatus. This supraneural will eventually fuse with arch 3 and become a component of the Weberian apparatus.

Stage 8

Stage 8 is marked by the completion of all chordacentra (i.e., mineralization of all protocentra). During this stage the compound centrum of the caudal fin, consisting of preural centrum 1 and ural centrum 1, forms before mineralization of the abdominal and preural protocentra is complete (Figs. 3B and C). Eventually, as the abdominal centra form, in an anterior to posterior direction, they catch up with the formation of the ural centra (Fig. 3D). Histological cross sections show that most chordacentra have thickened, and that the anterior neural arches and parapophyses are for the most part fused with their corresponding centra. This is achieved by perichondral ossification of the dorsal and ventral arcocentra and their fusion to the autocentra by a thin superficial ossification (Schultze & Arratia, 1992). Arcocentra and

autocentra in these vertebrae are now indistinguishable, and the vertebrae exhibit the adult condition (i.e., well-ossified structures developmentally formed both chondral and perichordal bone; Grizzle & Rogers, 1985).

Concurrently in series A and B, uroneural 1 forms at the distal margin of ural centrum 1. Interestingly, the formation of uroneural 1 in series A and B occurs later than in series C but at approximately the same TL, 11.5–13.6 mm. A lengthening of uroneural 1 to the distal margin of ural centrum 2 was observed in all series.

At this stage the second dorsal fin spine forms. In ictalurids the base of the second fin spine forms an ectoconvex surface that grips its cartilaginous proximal radial. When the radial ossifies, it fuses with the lepidotrichia and becomes the base of the definitive spine (Reed, 1924).

Although hypural and parhypural formation occurs early in development, ossification of the caudal fin supports occurs later. At this stage the parhypural begins to ossify at its midpoint in series A and B. This ossification began during stage 6 in series C. The parhypural, however, does not fuse to the compound centrum, and the distal end of this element does not ossify until much later.

Stage 9

Fishes at this stage exhibit a complete fusion of vertebral centra 2 and 3. The Weberian apparatus in ictalurids consists of ossified elements supported by a compound centrum that is a fusion of vertebrae 2, 3, and 4. These vertebrae fuse with each other sequentially. The fusion of these centra begins ventrally and ends with their final fusion at the dorsal margins.

Concurrently occurring features include ossification of all branchiostegal rays, the formation of teeth on the premaxilla, and the formation of cartilaginous distal radials in the anal fin in series A and B (occurring earlier, in stage 7, in series C).

Stage 10

Stage 10 is characterized by the articulation of the dorsal fin with the Weberian apparatus via neural spine 4. In ictalurids the proximal dorsal fin radials enlarge and expand ventrally, articulating with the spine of neural arch 4 of the Weberian apparatus. In adult specimens examined, this articulation is very strong, with virtually no play

between the bones. The functional significance of this is discussed in a subsequent section.

This stage also marks the appearance of the cartilaginous claustrum. The claustrum is the last of the Weberian ossicles to form and the last to ossify. Concurrent characteristics observed at this stage include an increase in number of teeth on the ventral and dorsal gill arches and the formation of five distal radials in the dorsal fin. This stage also marks the formation of a series of cartilaginous median elements associated with the posterior six free preural vertebrae (Fig. 3D). The formation of these elements has not been previously reported for *I. punctatus*. In the course of their development, each of the median elements will fuse undetectably to its corresponding neural spine. This condition and the possible homology of these elements with epurals are addressed further in the Discussion.

In the pectoral fins, the ventral ends of the scapulocoracoid cartilages continue to elongate and meet midventrally. Of the eight to nine fin rays present, the one on the leading edge of the fin has become thickened, and in series C the basal portion ossifies and forms small projecting hooks. In the pelvic fins, the small cartilaginous bars have expanded into triangular plates with seven to eight projecting rays.

Stage 11

Stage 11 is defined by the complete fusion of vertebra 4 with the combined vertebra of 2 + 3 to form the foundation of the Weberian apparatus. Although vertebra 5 is associated with the Weberian apparatus and partially articulates with it ventrally, as seen in many specimens examined (Al-Rawi, 1966), it is not considered part of the Weberian apparatus in ictalurids (Fink & Fink, 1981, 1996; Coburn & Grubich, 1998).

Additional characteristics observed at this stage include an elongation of the autogenous epural, the median elements associated with posterior neural spines, plus the formation of the basal segment of the pectoral fin spines in series A and B. In series C the basal ossification of the pectoral fin spine that began in stage 10 has extended distally to two-thirds of the spine. In series C, all of the elements of the pectoral girdle and fins are ossified except for a small central portion of the scapulocoracoid associated with the radials and the distal portion of the spine.

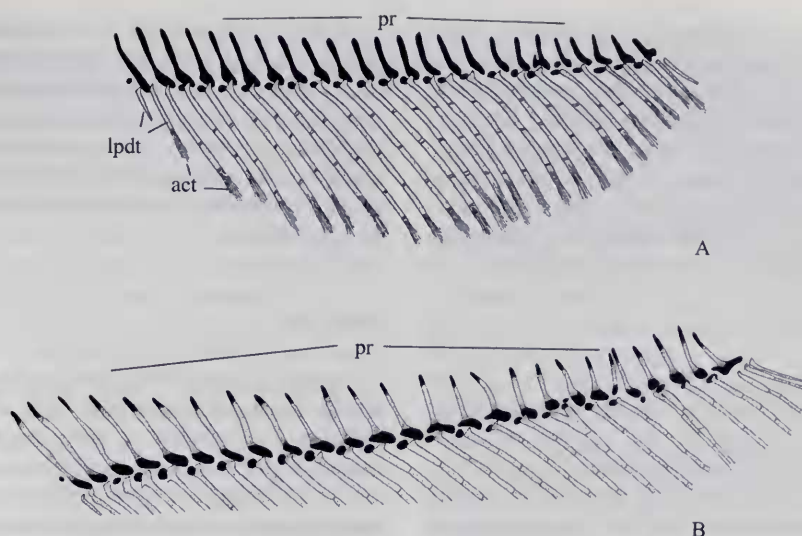


FIG. 4. Development of the anal fin skeleton. **A**, Foraging juvenile, 13 mm TL (stage 9). **B**, Adult, 30 mm TL (no stage). Anterior is to the left. For abbreviations, see p. 4.

Stage 12

Stage 12 is defined by the articulation of the anterior part of the transverse process of vertebra 4 (tp4) with the suspensorium of the pectoral girdle. Also in this stage the uroneural extends to the distal margin of the hypurals. Its length is complete, and in subsequent stages it will enlarge only in girth. All neural and hemal arches are ossified, although parapophyses posterior to hemal arch 9 are cartilaginous. In the skull, dermal bones enlarge in size and the lateral and medial walls of the hyomandibula begin to ossify. In series C, the first sign of ossification in the hyomandibula occurs much earlier, in stage 5. Teeth are present on ceratobranchial 5 of the ventral gill arches.

Stage 13

Stage 13 is defined by the complete ossification of all Weberian ossicles. The last ossicle to ossify is the claustrum. The two halves of the neural arch of vertebra 5 meet along the dorsal midline. The joining of these arches does not form a neural spine (there are no neural spines on arches 5–11). In fact, the only reason they are able to join is that neural arch 5 is always positioned between the elongated dorsal fin radials 2 and 3. In other words, the modified dorsal fin radials never interfere with the formation of this arch as they do

with the more posterior arches. The anterolateral processes of the pelvic basiptyrgia are formed.

Stage 14

All hypurals are at least two-thirds ossified. In most cases, only the distal rim of each hypural remains cartilaginous. This rim remains cartilaginous for a long time, and is still present in fish of 40 mm TL. In series C, hypurals 1–5 are ossified, except for the distal rims, but hypural 6 has only now reached two-thirds ossification. All posterior median elements associated with neural arches, which include the autogenous epural, are at least one-half ossified. Likewise, skull bones such as the opercular series, pterygoids, dentary, hyomandibula, supraoccipital, pterotics, and basioccipital, are ossified. The frontal bones continue to enlarge, and this enlargement is correlated with the development of the lateral line system. The caudal fin is now deeply forked, as is characteristic of the adult condition in this species.

Stage 15

At this stage the radials of the anal fin are well ossified (Figs. 4A and B). As stated previously, the middle radials are most likely lost in ictalurids. The proximal radial equivalent of these radi-

als ossifies first, followed by the middle radial equivalent. The distal radials of the anal fin ossify last (Fig. 4B). By this stage the pectoral girdle has ossified in series A and B (this event occurred in stage 11 in series C), and all but the last dorsal fin pterygiophore is bone. Hemal arches anterior to preural centrum 2 are fused with their corresponding centra, as is characteristic of most ostariophysans (Fink & Fink, 1981; Fig. 3G).

Stage 16

Stage 16 is defined by the completion of the Weberian apparatus dorsal fin complex. In addition, the once cartilaginous median elements attached posteriorly to the preural neural spines have fused completely with the corresponding spines in fish of 40 mm TL in series A and B. It is possible that this fusion occurred earlier in series C. In fish 40 mm TL and larger it is impossible to distinguish these elements from their neural spines. By this stage only one autogenous epural remains positioned dorsal to the compound caudal centrum. The fusion of posterior elements such as these with their neural spines strengthens the caudal fin and adds support for musculature (Lauder, 1989).

Stage 17

Hypurals 3 and 4 become articulated with ural centrum 2 and form a unit. According to Lundberg and Baskin (1969), ural centrum 2 never fuses with the compound centrum, but a fusion of ural centrum 2 and hypurals 3 and 4 does occur. We agree with Lundberg and Baskin (1969) that a structural unit does form between ural centrum 2 and hypurals 3 and 4, but we do not agree that a fusion of the three elements is always the case. We have found that in some cases, hypurals 3 and 4 fuse with each other, but in other specimens of the same size they only articulate. In the majority of the developmental material examined (specimens 50 mm TL and smaller), fusion of the three elements does not occur. It appears that only in very large specimens does such a fusion occur. It is possible that the fusion of hypurals 3 and 4 with each other and with ural centrum 2 is a very late developmental occurrence. We also have observed, although rarely (LU F082282), fusion of the ural centrum 2 unit with the compound centrum in series A.

In fish about 45 mm TL, the skulls are completely ossified, although the right and left frontal bones still do not suture completely in the dorsal midline. The basipterygia of the pelvic fins are beginning to ossify. The last structures to ossify are the distal radials of the dorsal and anal fins. At this stage the development of the lateral line is complete.

Stage 18

In stage 18, the compound centrum in the caudal fin forms, consisting of preural centrum 1, ural centrum 1, uroneural 1, and parhypural and hypurals 1 and 2. This is the last stage in the development of the postcranial skeleton. This stage occurs quite late in development and is seen only in fish longer than 50 mm TL. Also in this stage all hemal spines anterior to preural centrum 2 fuse with their corresponding centra. Fishes at this stage exhibit adult pigmentation.

Discussion

In this section the development of the postcranial skeleton of *I. punctatus* is examined in discrete developmental units—the median fin folds, the pectoral and pelvic fins, the Weberian apparatus/dorsal fin complex, and the caudal fin skeleton. The results of our study are discussed in conjunction with the findings of other researchers in an attempt to better understand the development of the postcranial skeleton in ictalurids. Debates and controversies involving the homologies of certain skeletal structures (e.g., the anterior nuchal plate) are addressed. Finally, we discuss the variation within and between each of the developmental series examined. An understanding of variation is key to developmental studies such as this one, and to anatomical studies in general.

Overview of Median Fin Fold Formation and Development

The sequence of median fin formation and the order of development of their constituent radials, rays, and spines varies among actinopterygians (Dunn, 1983). The median fins of *I. punctatus* form from a thin continuous ridge or fold that extends from behind the head around the posterior

tip of the notochord and ventrally to the anal opening. No secondary fin folds are present. By the end of the yolk sac period all median fins have formed, and the fins resemble their adult shapes. Interestingly, the sequence of median fin formation does not parallel the sequence of skeletal support formation of these same fins. In other words, the first fin lobe to form is the dorsal, followed by the caudal and then the anal. The first fin supports to form, however, are in the caudal fin, followed closely by those in the dorsal fin and then in the anal fin. The sequence of fin formation in *I. punctatus* differs from the sequences described for paddlefishes (a lower actinopterygian) by Bemis and Grande (1999) in that an anterior to posterior direction of fin formation was not observed. On the other hand, the fin formation sequence in *I. punctatus* directly parallels that described by Fuiman (1983) for other ostariophysans (e.g., *Hypentelium etowanum*), and for esocoids and scombroids as described by Martin (1983) and Collette et al. (1983), respectively. It is possible that the sequence of fin formation seen in *I. punctatus* is typical for ostariophysans and possibly teleosts in general, but not for lower actinopterygians.

Figure 5 illustrates the formation of the median fins and skeletal supports in four developmentally different specimens of *I. punctatus* from series A and B. Although specimens of series C followed the same sequence of defining criteria as series A and B, they were generally larger when they achieved each stage (see Table 1). In embryonic fishes about 7 mm TL (not illustrated), the first fin supports to form (i.e., the parhypural and hypurals 1–2) are in the caudal region below the upturned notochord. The notochord extends almost to the posterodorsal margin of the continuous median fin fold. No other fin supports are present at this time. In embryonic fishes about 10 mm TL (Fig. 5A), the dorsal fin fold begins to appear, and seven dorsal radials are discernible. A distinct caudal fin lobe is not evident at this time, although one autogenous epural is formed above the notochord, and five hypurals with at least 13 fin rays are present below it. On the ventral side, 18 anal fin radials have formed in an anterior to posterior direction in fishes of this size, even though no anal fin has formed. The median fin fold now appears to be discontinuous between the dorsal fin and the remainder of the fin fold. In fishes about 11.5 TL (Fig. 6A) the dorsal fin has enlarged and fin rays are present in a one-to-one pattern with their corresponding radials. Ural centrum 1 is formed in the caudal fin. By the time

the now yolk-sac larval fish have reached about 12–14 mm TL (Figs. 5B and 6B), the dorsal radials have elongated toward the developing Weberian apparatus, all caudal fin skeletal supports are formed in cartilage, the caudal fin rays are segmented, and the fin begins to take on its characteristic forked appearance. In the anal fin, the proximal radials have elongated and the distal radials begin to form. Also in this stage the adipose fin forms. Remnants of the median fin fold are present between the adipose and caudal fins. From this point on (foraging period), as illustrated in Figures 5C and D and 6C and D, median fin development is devoted to the ossification of the skeletal supports, the formation of the dorsal fin spines, and the forking of the caudal fin. In fishes 25 mm TL (Fig. 5D), no sign of the median fin fold remains.

The formation of the median fins in *I. punctatus*, and possibly other ostariophysans, seems to be functionally correlated. The caudal fin supports, first to form, are necessary for locomotion and are possibly involved in propulsive escape maneuvers from predators. The dorsal fin and its spines, second in the series to develop, aid in the defense of the fish. Both fins enhance the survivability of the free-swimming yet immature fish and thus are strategically important to form first, before the anal and pelvic fins.

Overview of Paired Fin Development

The formation of the median fins is clearly independent of the formation of the pectoral and pelvic fins. The pectoral fin buds appear first in fishes about 6.8 mm TL (Fig. 1A). They are not attached to the body but appear to emerge from the yolk sac covering. As the fish's body increases in height the yolk sac gets smaller and the pectoral fin buds move closer to the sides of the body, eventually to become part of the flank when the yolk sac is depleted and the ventral body wall forms. Based on the development of series C, internal skeletal support begins with the formation of the scapulocoracoid cartilage posterior to the cleithrum in specimens of 8.5–10 mm TL. Posterior to the scapulocoracoid is a layer of cartilage that will form three radials when the fishes reach 10–12 mm TL. The anteriormost radial forms as a part of the scapulocoracoid cartilage; the other two are separate. As the larvae exceed 12 mm TL, the six or seven fin rays present begin to ossify, and the scapulocoracoid cartilage elongates dor-

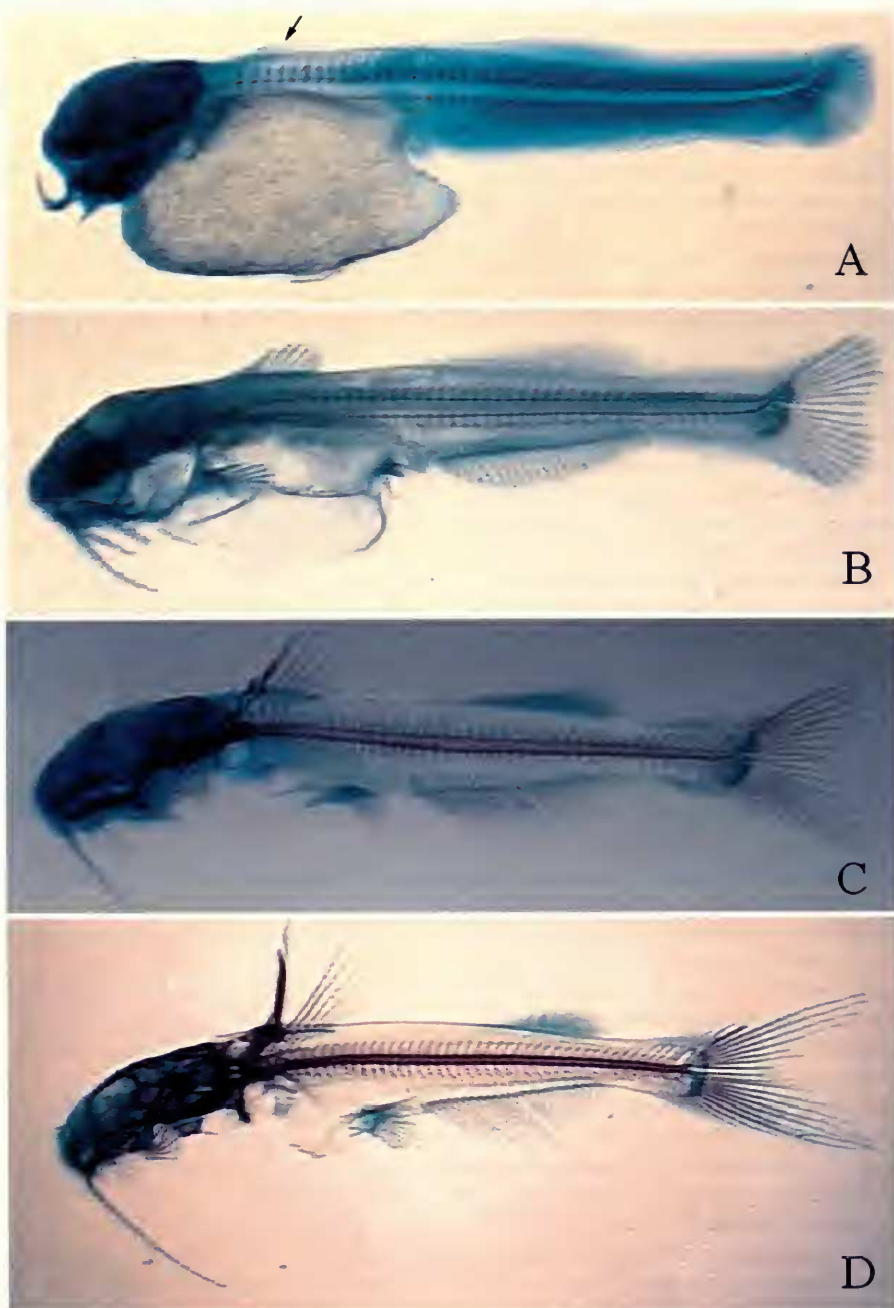


FIG. 5. Series of cleared and double-stained specimens showing the development of the median fins and postcranial axial skeleton. Arrows point to the formation of the median fins. **A**, Embryonic larvae, 10 mm TL (stage 3–4). **B**, Yolk sac larvae, 15.2 mm TL (stage 11–12). **C**, Foraging juvenile, 15.2 mm TL (stage 12). **D**, Foraging juvenile, 25 mm TL (stage 14–15).

sally and anteroventrally along the medial side of the cleithrum. In specimens of 13–16 mm TL, the coracoid portions have elongated sufficiently to meet midventrally. The anteriormost fin ray has a

thick ossified basal portion and will become the spine. This spine becomes two-thirds ossified and has projecting hooks as larvae increase to 17.5 mm TL. At this point ossification has begun in all

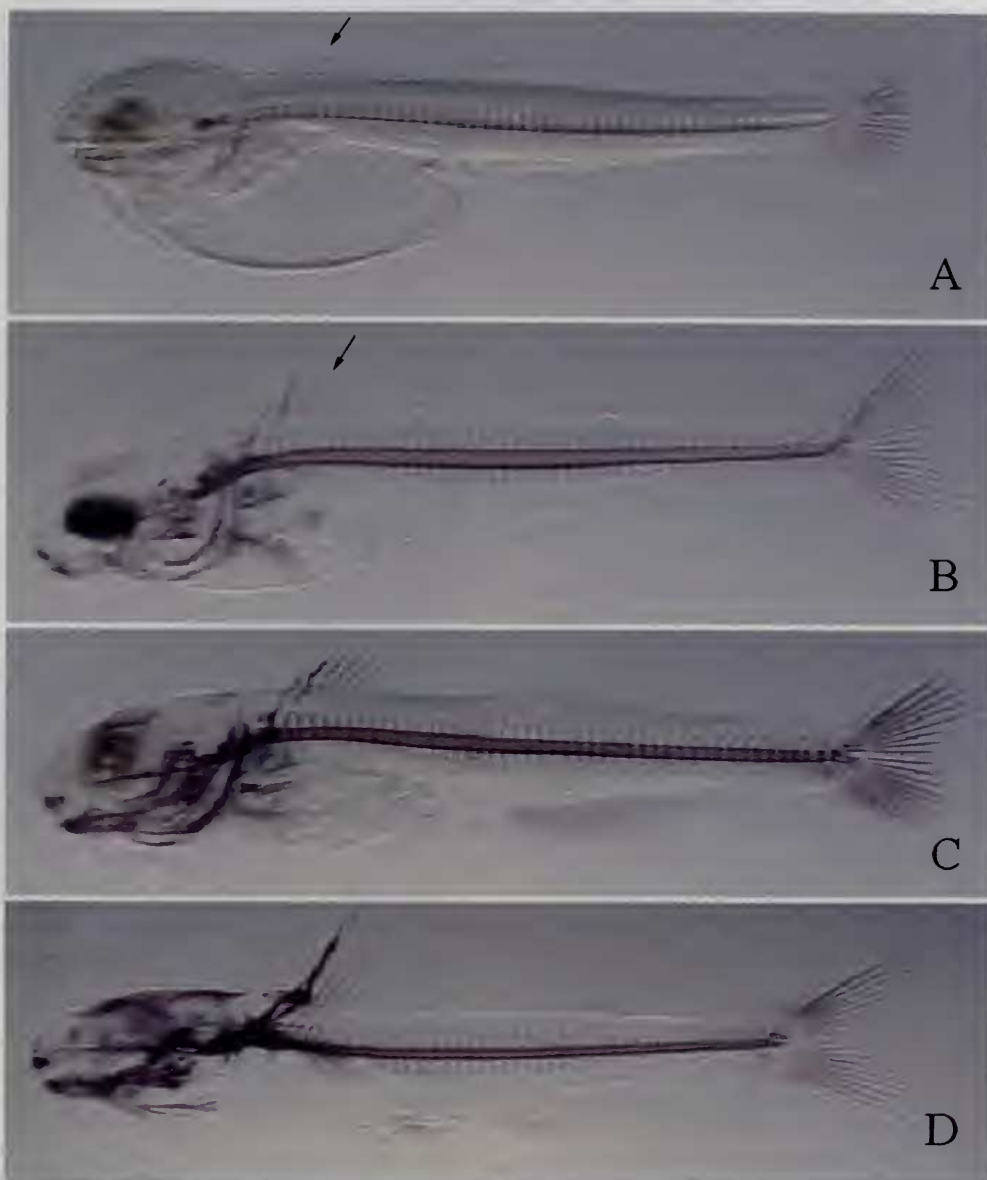


FIG. 6. Series of alizarin-stained specimens showing the development of the vertebral column, median fin supports, and dermal skull bones. Arrows mark the dorsal fin fold. **A**, Yolk sac larvae, 11.5 mm TL (stage 4). **B**, Yolk sac larvae, 13.7 mm TL (stage 8). **C**, Foraging juvenile, 19 mm TL (stage 14). **D**, Foraging juvenile, 23.5 mm TL (stage 15).

of the pectoral girdle and fins except for a small portion of the scapulocoracoid cartilage associated with the radials and tips of the radials.

Pelvic fins form later in development, when the larvae are about 8.5–10 mm TL (series C). Pelvic fin buds form along two membranous creases formed by the attachment of the posterodorsal portion of the yolk sac to the body. The buds lie

lateral to the fin fold and just anterior to the anal opening. By the time the fishes have reached 12 mm TL, the basipterygia have condensed as small cartilage rods, which further expand into triangular shapes as the fishes grow to 16–17.5 mm TL. Seven to eight fin rays form, and both fin rays and basipterygia are ossified in specimens 32 mm TL and larger.

Development of the Caudal Fin Skeleton

Our observations of the development of the caudal fin skeleton of *I. punctatus* show some fundamental consistencies in the three series examined, as well as considerable individual variation between and within the series. Our observations differ from those of Lundberg and Baskin (1969), who found little individual variation among the specimens of *I. punctatus* they examined. In this study we highlight the different types of caudal skeleton variation observed but will focus on the general developmental patterns found in all three series. The sizes apply to series A and B.

As discussed in François (1966), Monod (1968), Laerm (1976), Schultze and Arratia (1989), Arratia and Schultze (1992), and Grande and Bemis (1998), the first vertebral elements to form in actinopterygians are the cartilaginous dorsal and ventral arcocentra (*sensu* Arratia & Schultze, 1992). From within a constricted notochord, chordocentra form, followed by the perichondral ossification of the arcocentra and their fusion with the autocentra, which in turn surround the chordocentra. In *I. punctatus*, the arcocentra (i.e., neural and hemal arch precursors) have formed in conjunction with the parhypural and hypurals in fishes about 7 mm TL (Fig. 3A, stage 2). The protocentra do not begin to mineralize until the fish reaches about 9–10 mm TL. As described earlier in the discussion of stages 4, 5, and 6, mineralization of the protocentra begins in the anterior region of the vertebral column, just behind the skull, and continues posteriorly. Before the mineralization process reaches the posterior centra, however, the first ural centrum begins to form along its ventral margin (Fig. 3B), associated only with hypurals 1 and 2. As ural centrum 1 continues to develop, it enlarges and, in specimens of about 11 mm TL, is attached to preural centrum 1 (Fig. 3C). Interestingly, in no specimen examined was an autogenous preural centrum 1 observed. As illustrated in Figures 3E and F, some of the cleared and stained specimens show vertical demarcations within the compound centrum, presumably between preural centrum 1 and ural centrum 1. We infer from these specimens, and agree with Lundberg and Baskin (1969), that the compound centrum is formed from two protocentra (i.e., preural centrum 1 and ural centrum 1 pu1 + u1) that have developed together, with ural centrum 1 forming first. Ural centrum 2 remains associated with hypurals 3 and 4, and only in a few

specimens examined became fused into the compound centrum.

In specimens of 13 mm TL, a thin, single uroneural (un1) has formed and is fused to the posterodorsal margin of the compound centrum (Fig. 3D). Uroneural 1 has a wavelike appearance at this point, but it will become characteristically rigid as it enlarges. Although uroneurals in primitive teleosts are preformed in cartilage (Schultze & Arratia, 1989; Arratia & Schultze, 1992), we found that uroneural 1 in *I. punctatus* develops from membrane bone. Additionally, Arratia (personal communication, 2000) found that in specimens of *I. punctatus* that she has examined, ossification of uroneural 1 begins caudally and continues anteriorly, surrounding the developing ural neural arch, and later fuses with the compound centrum. We were not able to corroborate her observations. In our developmental material it appears that uroneural 1 begins to form rostrally. In specimens of increasing size and age, uroneural 1 increases in length and eventually reaches the posterior margin of hypural 6 (e.g., specimens of 20 mm TL). It is likely that variation in uroneural formation occurs, but, in the specimens we examined, uroneural 1 does not appear to begin its development caudally and ossify rostrally. Other caudal fin characteristics include the presence of an enlargement of ural centrum 2, and the first sign of bone in the parhypural.

The siluriform caudal skeleton as described by Lundberg and Baskin (1969) has a single epural positioned above the neural arch of pu1 + u1. We found that this is true for adults, but the adult condition has a complex ontogenetic origin in *I. punctatus*. We observed the formation of six cartilaginous median elements positioned and usually attached to the posterior edge of the posteriormost neural spines (Figs. 3 and 7). Although in most specimens examined these elements are loosely attached posteriorly to the neural spines, in LU F.082278 and LU F.082279 two and three autogenous elements were observed (Figs. 3D and E). We recognize that the condition in these specimens may be an anomaly. On the other hand, it may indicate separate ossification centers for these elements which differ from the ossification centers of the neural arches or spines. During development, all of these elements elongate, ossify, and completely fuse with their corresponding neural spines (Figs. 3E–G and Fig. 7). With the exception of the last epural in the adult channel catfish, there is no indication that cartilaginous elements were ever associated with posterior neural

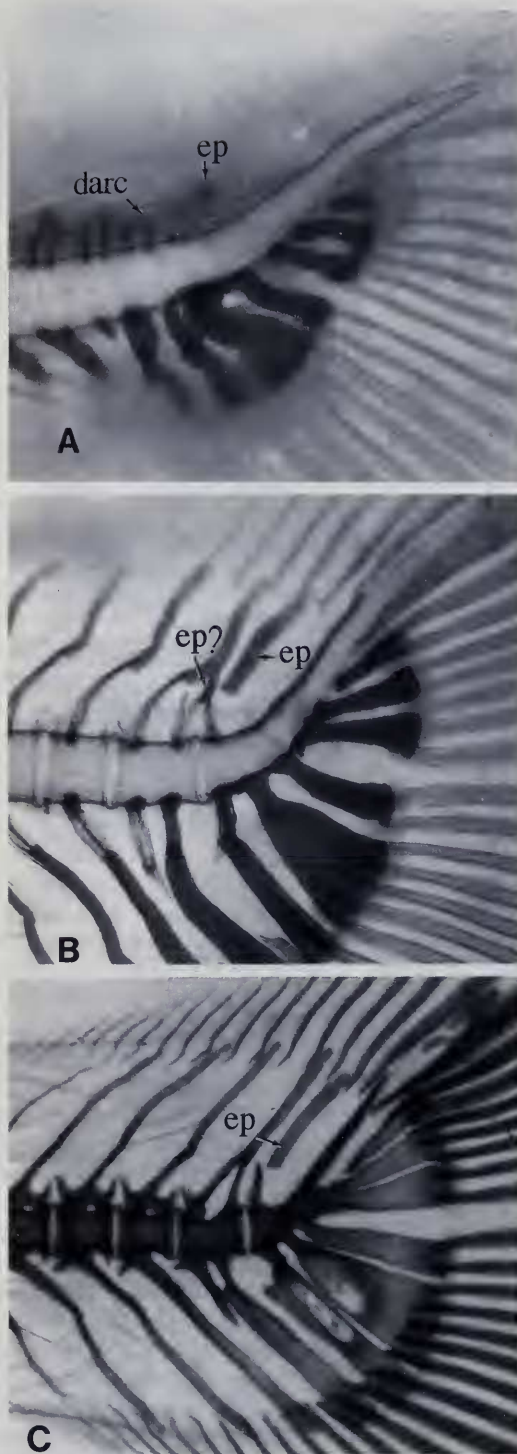


FIG. 7. Photograph of cleared and stained specimens showing the formation of the autogenous epural and the epurals associated with neural spines. A, Yolk sac larvae, 11.0 mm TL (stage 4–5). B, Yolk sac larvae, 15

spines. The homology of these elements is debatable, but, based on the development of these elements (i.e., as median elements that elongate and ossify in the same pattern as the autogenous epural) and their placement in the body plane (i.e., in sequence with the autogenous epural), we entertain the possibility that these elements are serial homologues of the autogenous epural (Monod, 1968). We acknowledge that epurals associated with preural centra 3–6 have not been reported in ostariophysans, but we argue that until a more thorough survey of skeletal development in primitive teleosts is conducted, this interpretation cannot be ruled out. At the very least, the “epural” condition in *I. punctatus* may provide insight into the much debated origins and homologies of epurals. As defined by Goodrich (1930), epurals are modified radials. Schultze and Arratia (1989) and Arratia and Schultze (1992) argue that epurals are detached neural spines, while Patterson (1968) and Grande and Bemis (1991) argue that epurals are serial homologues of supraneurals. In *I. punctatus*, these structures are clearly associated with neural spines. The more anterior ones abut and eventually become incorporated into their spine, while the autogenous epural is positioned directly above a stunted neural spine of the compound centrum in the caudal skeleton. With this said, we also observed that these median structures form as cartilaginous condensations after the formation and almost complete ossification of their corresponding neural spines. In other words, the formation of these elements seems to be secondary to the formation of neural spines, and to occur from separate ossification centers. As discussed by Arratia and Schultze (1992), epurals form in different ways, and those that arise from independent cartilages can be interpreted as neural spines that have lost their arches. If their interpretation is correct, then our observations would support the connection between epurals and neural spines.

As the ossification of the caudal fin skeleton continues (Fig. 3E), the “epurals” elongate and ossify as discussed above, the hypurals ossify except at their distal margins, and hypurals 1 and 2 begin to show signs of fusion with the compound

←
mm TL (stage 11). C, Foraging juvenile, 26.3 mm TL (stage 16). Note that in B, a second autogenous epural is present (marked by an arrow), corresponding to the neural spine of preural 2 (pu2) (LU F082289). For abbreviations, see p. 4.

centrum. Uroneural 1 has elongated, extending past the distal edges of the hypurals. Internally (as seen only in cleared and stained material), specimens of about 20 mm TL still show an articulation line between *pu1* and *u1* (Figs. 3E and F). In specimens of 20–50 mm TL, hypurals 3 and 4 are separate, and although they are in contact with ural centrum 2, they are not fused with it. Figure 3F shows that ural centrum 2 forms a cap around the base of the articulating hypurals 3 and 4 in fishes of about 40 mm TL. As reported by Lundberg and Baskin (1969) and Fink and Fink (1981), ural centrum 2 is fused with the bases of hypurals 3 and 4 in most siluroids (in Diplomystidae, hypurals 3 and 4 remain separate elements: Arratia, 1987). This unit in turn articulates with but never fuses with the compound centrum. Our observations corroborate these findings in most specimens examined (Fig. 3G). Our results indicate, however, that the caudal fin skeleton continues ossifying, and that additional fusions of skeletal elements occur long into the adult stage. Although the fusion of hypurals 3 and 4 with each other and with ural centrum 2 seems to be the common condition, we have also found variation in comparably sized adult specimens in which hypurals 3 and 4 never fuse with ural centrum 2. Ural centrum 2 remains as a cap around the bases of hypurals 3 and 4, as illustrated in Figure 3F. In other specimens examined, hypurals 3 and 4 do fuse with ural centrum 2, and ural centrum 2 fuses into the compound centrum (e.g., LU F082282).

An additional caudal fin anomaly observed and worth reporting is the amount of intraspecific variation in hypural number (Fig. 8). Intraspecific and interspecific variation in hypural loss and fusion has been reported by Lundberg and Baskin (1969) and Arratia (1983), and although interspecific variation is common in catfishes, intraspecific variation in hypural number is rare. Lundberg and Baskin (1969) found considerable intraspecific variation in a population of *Noturus gyrinus*, which they argued is the result of hypural fusions, but they found none in the 13 species of *Ictalurus* they also examined. Arratia (1983) found considerable interspecific variation in hypural number within Trichomycteridae (subfamily Trichomycterinae), but found intraspecific variation only in the Andean trichomycterids (e.g., *Trichomycterus laucaensis*).

Ictalurus punctatus specimens of about 10 mm TL and smaller had only five or fewer hypurals. If a sixth hypural forms, it forms in fishes of more than 11 mm TL. Although ictalurids are diag-

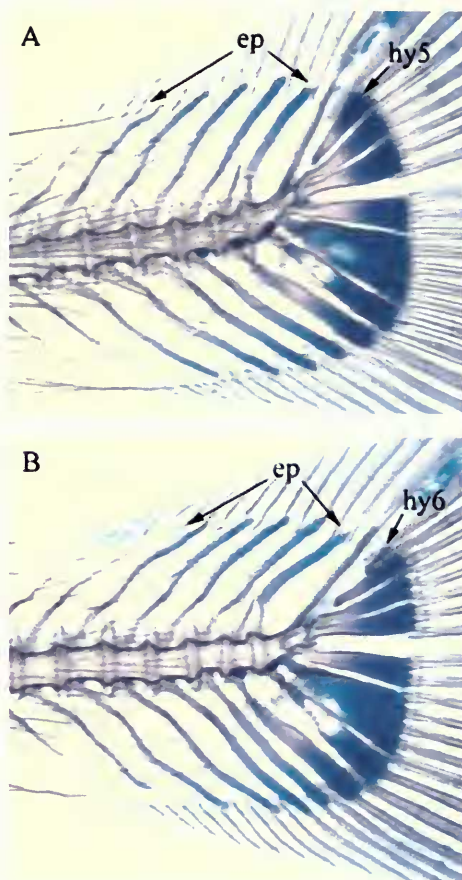


FIG. 8. Cleared and stained specimens showing variation in the number of caudal fin hypurals. A. Adult, 30 mm TL, with five hypurals (LU F082284). B. Adult, 27 mm TL, with six hypurals (LU F082283).

nosed in part by the presence of six hypurals (Lundberg & Baskin, 1969; Nelson, 1994, p. 00), over one-third of the specimens we examined in our developmental series had only five. We discount the possibility of early hypural fusions as an explanation for this phenomenon and agree with Lundberg and Baskin (1969) that hypural fusion does not necessarily take place with increasing size. We also think that it is hypural 6 that has been lost, because when a hypural is absent, it is always in the position of the sixth, which is the last of the series to develop. In no specimen examined with five hypurals were rudiments of the sixth hypural observed, and in no specimen with six hypurals were hypurals 5 and 6 ever fused or partially fused. The occurrence of five hypurals instead of six is widespread among all three developmental series examined and, like the

different observed configurations of hypurals 3 and 4 with ural centrum 2, seems to be random. We therefore suggest caution when using hypural number as a caudal fin character to diagnose *I. punctatus*. It is possible that the number of hypurals may not be a reliable character to diagnose species within Ictaluridae.

Development of the Weberian Apparatus/ Dorsal Fin Unit

The Weberian apparatus, diagnostic of the Oto-physi (Rosen & Greenwood, 1970), consists of a series of modified anterior centra, neural arches, and pleural ribs that connects the gasbladder to the back of the skull. When the gasbladder pulsates in a sound field, high-frequency vibrations are transmitted from it via the Weberian ossicles to the back of the skull and then to the inner ear (Alexander, 1964, 1965). This system, along with the lateral line system, enables the fish to receive a wide range of sound frequencies. Although the Weberian apparatus is assumed to function in the same basic way among otophysan subgroups, morphological variation among these subgroups is apparent. This variation, or possible specialization, is exemplified by the Siluroidei and includes, at the primitive level, a fusion of vertebral centra 2–4, loss of the articular process of the intercalarium, and modifications of the tripus, os suspensorium, and the transverse process of the fourth centrum (Fink & Fink, 1981, 1996).

The Weberian apparatus of *I. punctatus* and many other catfishes is functionally complex because of its close association with the dorsal fin skeleton (Chardon, 1968). In *I. punctatus*, the anterior two proximal radials of the dorsal fin are expanded, elongated, and tightly articulated with the neural spine of the fourth vertebra of the Weberian apparatus. In adult *I. punctatus*, the Weberian apparatus and the dorsal fin skeleton essentially form an interconnected unit whose development can be considered together; this in turn poses some interesting functional questions.

This study begins with the first appearance of identifiable Weberian ossicles in catfishes of about 10 mm TL (stage 6, series A and B). At this point in development, all of the basidorsals (i.e., dorsal arcocentra) have formed along the vertebral column. Basiventrals (i.e., ventral arcocentra) posterior to the second centra have also formed. Basiventrals 1 and 2 are absent in siluroids (Fink & Fink, 1981). Hypotheses concerning the deriva-

tion and homologies of Weberian ossicles are many. Watson (1939), Bamford (1948), and Rosen and Greenwood (1970) argue that the scaphium is derived from basidorsal 1 that consists of two processes, one dorsally oriented and another anteriorly oriented. Matveive (1929), Rademaker et al. (1989), and Vandewalle et al. (1990), however, argue that the scaphium is formed from basidorsal 1 plus an ossification of mesenchyme. Lal Hora (1922) states that cartilages from the skull contribute to the formation of the scaphium. Basidorsal 2, according to most authors (e.g., Watson, 1939; Fink & Fink, 1981; Vandewalle et al., 1990; Chardon & Vandewalle, 1997), will become the intercalarium, which at this point in the development of *I. punctatus* remains relatively indistinct, as are basidorsals 3 and 4. The claustrum, which some researchers argue is derived from neural arch 1 (e.g., Fink and Fink, 1981), or is derived from mesenchyme (e.g., Watson, 1939), or is homologous with the first supraneural (e.g., Gayet, 1982; Coburn & Futey, 1996), is not present in *I. punctatus* at this time.

Basiventral 3 and possibly the third pleural rib form the tripus (Fink & Fink, 1981, 1996). In *I. punctatus*, the tripus consists of a dorsal part that will extend anteriorly and, when fully formed, will flatten to articulate with the intercalarium. The more ventral part of the tripus, the transformator process, is long and threadlike in 10-mm fish. It will form a crescent-like structure on the ventral side of the complex centrum of the Weberian apparatus and become embedded in the tunica externa of the gasbladder. Basiventral 4 develops at a rapid rate. It is already thicker and more elongate relative to the other basiventrals. It will form the transverse process of the fourth centrum, and its anterior projection will articulate with the suspensorium of the shoulder girdle (Lundberg, 1975). The os suspensorium, although not present until later in development, forms from the ventral side of the transverse process and is embedded in the gasbladder.

The first signs of ossification in the Weberian ossicles occur in fish of about 12 or 13 mm TL (Fig. 9A). The first ossicle to begin ossifying is the tripus (i.e., dorsal component), followed by the leading tip of the transverse process of basiventral 4. The transformator process of the tripus and the proximal end of the transverse process ossify later. Basidorsal 4 has grown considerably larger than the other basidorsals, and its dorsal tip begins to bend posteriorly, approaching the first proximal radial of the dorsal fin. Basidorsals 3 and

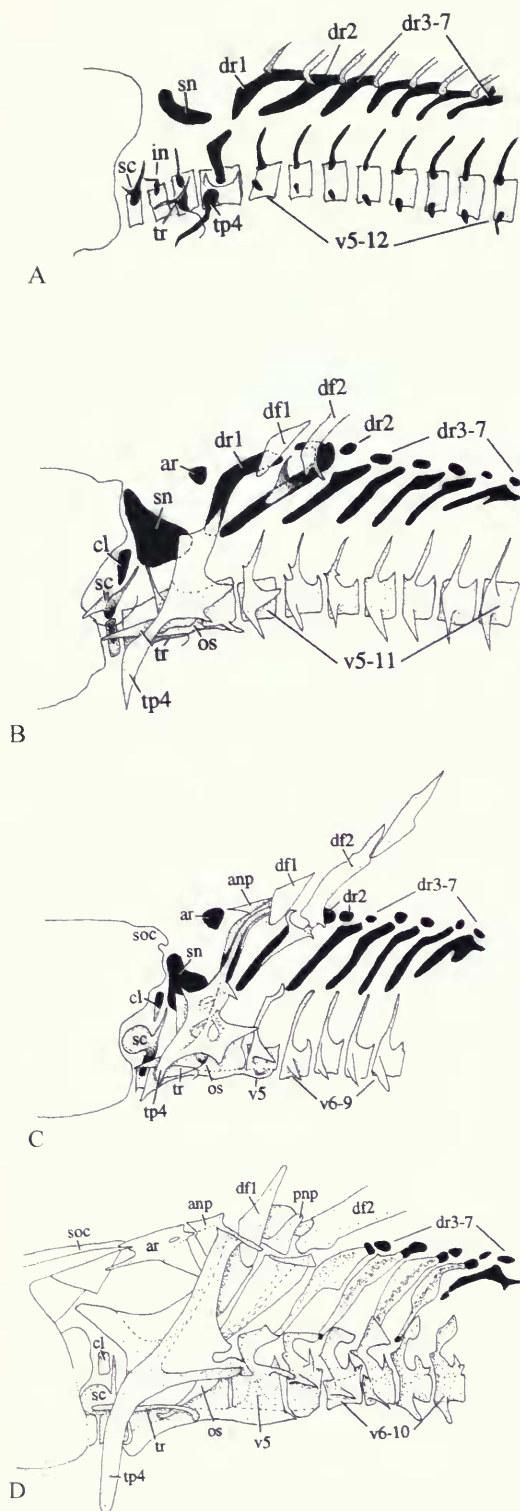


FIG. 9. Development of the Weberian apparatus/dorsal fin unit. A. Yolk sac larvae, 12.3 mm TL (stage 7) (LU

4 will form neural arches that join to an enlarged supraneural that projects forward and articulates with the ventral part of the supraoccipital (Fink & Fink, 1996). This large supraneural forms above vertebrae 2 and 3 and is present in fish of about 11 mm TL. Although in *I. punctatus* it initially forms as one crescent-shaped structure (Fig. 9A), some researchers argue for multiple ossification centers for this element (e.g., Coburn & Futey, 1996). Gayet and Chardon (1987) and Chardon and Vandewalle (1997) suggest that this supraneural represents a combination of supraneurals 2 and 3. Arratia (1987) argues that in *Diplomystes chilensis* this supraneural is actually formed from supraneurals 3 and 4, and that in specimen MNHN B. 584, these supraneurals were observed as separate elements (Arratia, 1987; Fig. 9). Our study was not able to shed light on this interpretation. Like Rosen and Greenwood's (1970) study of *Brycon meeki*, we observed only one center of ossification in all relevant specimens of *I. punctatus* examined. At this stage in development (about 11 mm TL), all of the vertebrae are autogenous.

In the dorsal fin, seven cartilaginous radials are formed. Although the first two radials will become incorporated into the Weberian apparatus/dorsal fin complex, the dorsal fin is completely separate from the basidorsals at this point. The first and most proximal segment of lepidotrichia has formed in the dorsal fin. Of these dorsal fin rays, the anterior two will develop into fin spines. Catfish spines form first as soft rays in discrete lepidotrichial segments. This is followed by the addition of concentric layers of dermal bone over the surface. The addition of these bony deposits causes the margins of these segments to gradually approach each other, and finally to fuse (Reed, 1924; Fig. 10).

In fishes of about 15–18 mm TL, the tripus is ossified and its anterior process has elongated to

F082285). B. Yolk sac larvae, 13.6 mm TL (stage 9–early stage 10), showing the connection between the first two dorsal fin radials and neural arch 4 (LU F082286). Also note that “anterior radial” (ar) is new terminology to reflect the serial homology of this element with the other dorsal fin radials. C. Foraging juvenile, 16.3 mm TL (stage 13), showing further development and the formation of the dorsal fin spines (LU F082287). D. Foraging juvenile, 38.5 mm TL (no stage), showing a connection with the back of the skull via the anterior radial and the Weberian apparatus (LU F082288). Anterior is to the left. For abbreviations, see p. 4.

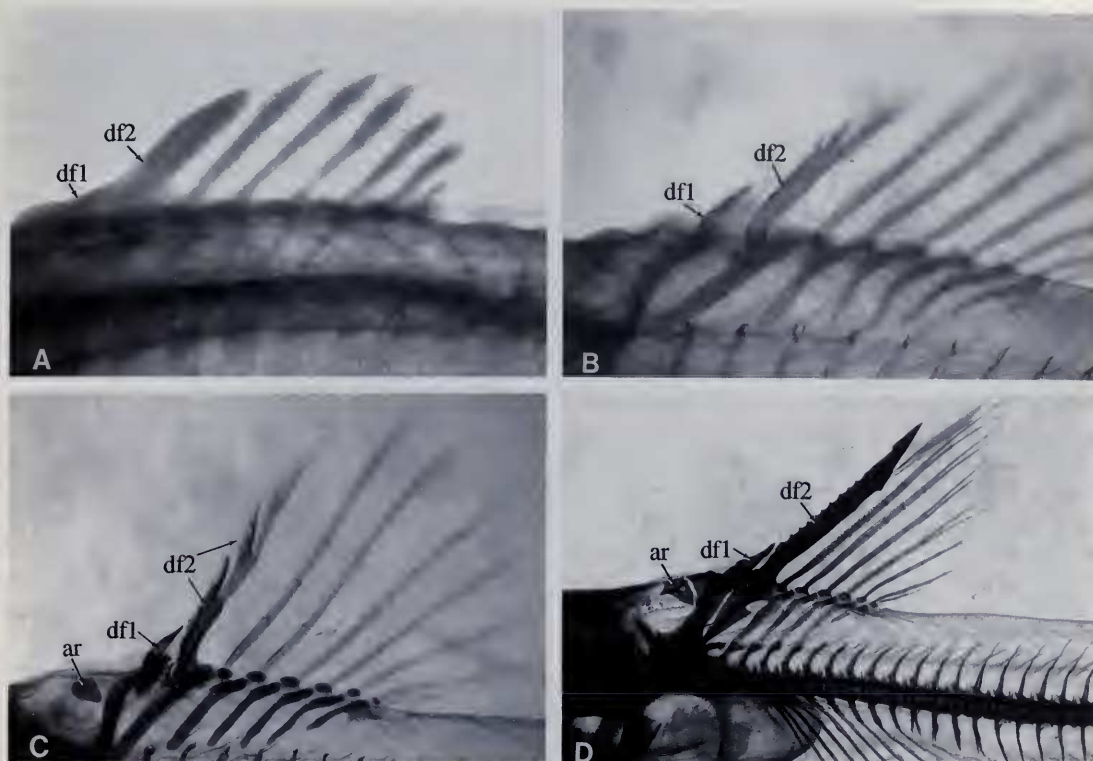


FIG. 10. Development of the first and second dorsal fin spines. A, Yolk sac larvae, 11.5 mm TL. B, Yolk sac larvae, 13.0 mm TL. C, Foraging juvenile, 17 mm TL. D, Foraging juvenile, 25.6 mm TL. For abbreviations, see p. 4.

the anterior margin of vertebra 1. The transverse process of vertebra 4 is also ossified and continues to expand (Fig. 9B). The os suspensorium has begun to form from the ventral side of the transverse process, near its connection of vertebra 4 with vertebra 5, starting with the dorsal lamina (Al-Rawi's terminology, 1966). The dorsal lamina extends anteromesially between the transformator process of the tripus and the postcardinal vein, and terminates in the radial nodule. The radial nodule in the adult catfish is surrounded by the crescent-shaped transformator process of the tripus. Both the dorsal lamina and the radial nodule are homologous with the fourth pleural rib and parapophysis of vertebra 4 (Fink & Fink, 1981). Additionally, the intercalarium is completely ossified, and the scaphium begins to exhibit its characteristic shape as its horizontal process extends toward the exoccipitals. The large supraneural (often called the neural complex; Rosen & Greenwood, 1970) has enlarged and, although still cartilaginous, articulates with the expanded neural arches of vertebrae 3 and 4. Also by this time (stage 11) vertebrae 2–4 are fused, thus complet-

ing the formation of the complex centrum. The fusion of vertebrae 2 and 3 occurs in fish averaging 13 mm TL (stage 9). Vertebra 1 remains independent and never fuses with the Weberian complex centrum.

In fishes of about 15 mm TL, the cartilaginous claustrum is seen for the first time above the scaphium (Fig. 9B). The homology and derivation of the claustrum have been debated recently. Fink and Fink (1981) hypothesized that the claustrum forms from a dissociated dorsomedial portion of the first neural arch. Coburn and Futey (1996) argued that the claustrum is derived from supraneural 1. Their hypothesis is based on their examination of several cyprinids (e.g., *Luxilus*) and catostomids (e.g., *Ictalobus*), in which supraneural 2 appears to form early in development from paired structures, fusing later in development. They argue that because supraneural 2 forms as a paired element, so might supraneural 1, and if that is true, then it is conceivable that the claustrum forms from supraneural 1. They also found no evidence for a dorsal extension of neural arch 1. Although our observations, based on histology

and cleared and stained specimens of *I. punctatus*, cannot support Coburn and Futey's (1996) observations of paired supraneurals, we also cannot support the hypothesis that the claustrum forms from neural arch 1, as does the scaphium. The claustrum in *I. punctatus* is the last Weberian ossicle to form. It appears in cartilage after the scaphium has ossified, and we can see no dorsal extension connecting the scaphium with the claustrum, or any claustrum precursor, at any time during development. It is possible that if supraneurals begin development as paired structures, as Coburn and Futey (1996) argue, then they very quickly fuse, resulting in median elements. If this happens, then we may have missed our small window of opportunity to observe this supraneural forming from essentially two halves in the specimens observed. Additional ontogenetic material of other siluriforms and closer sampling times may be necessary to resolve this.

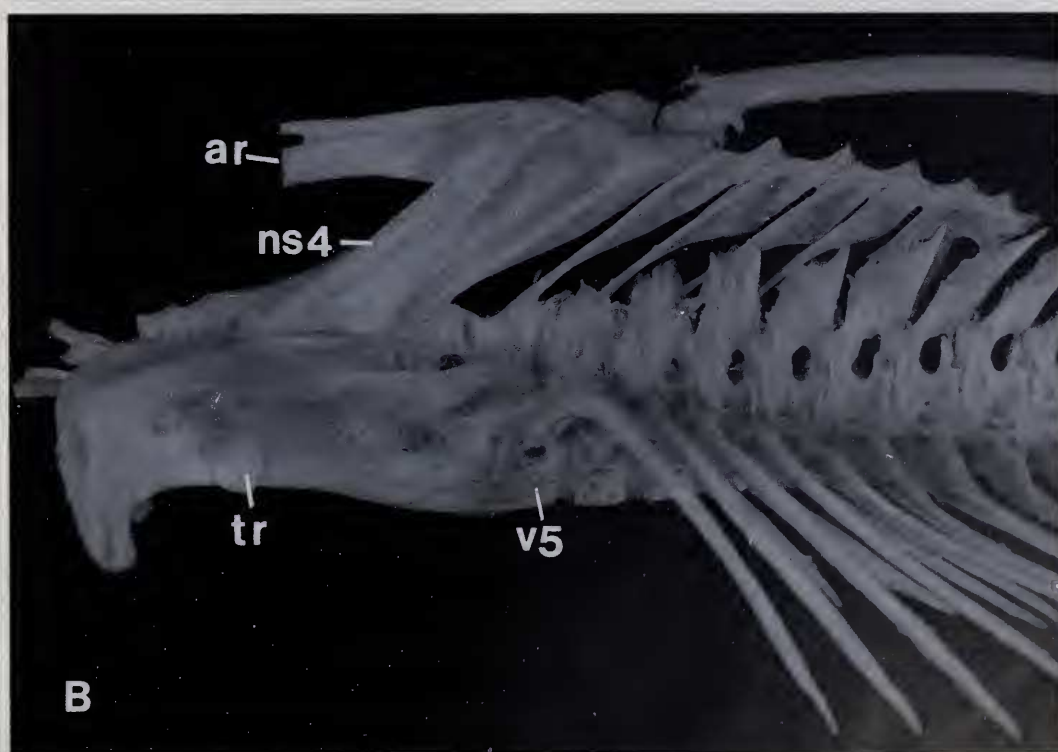
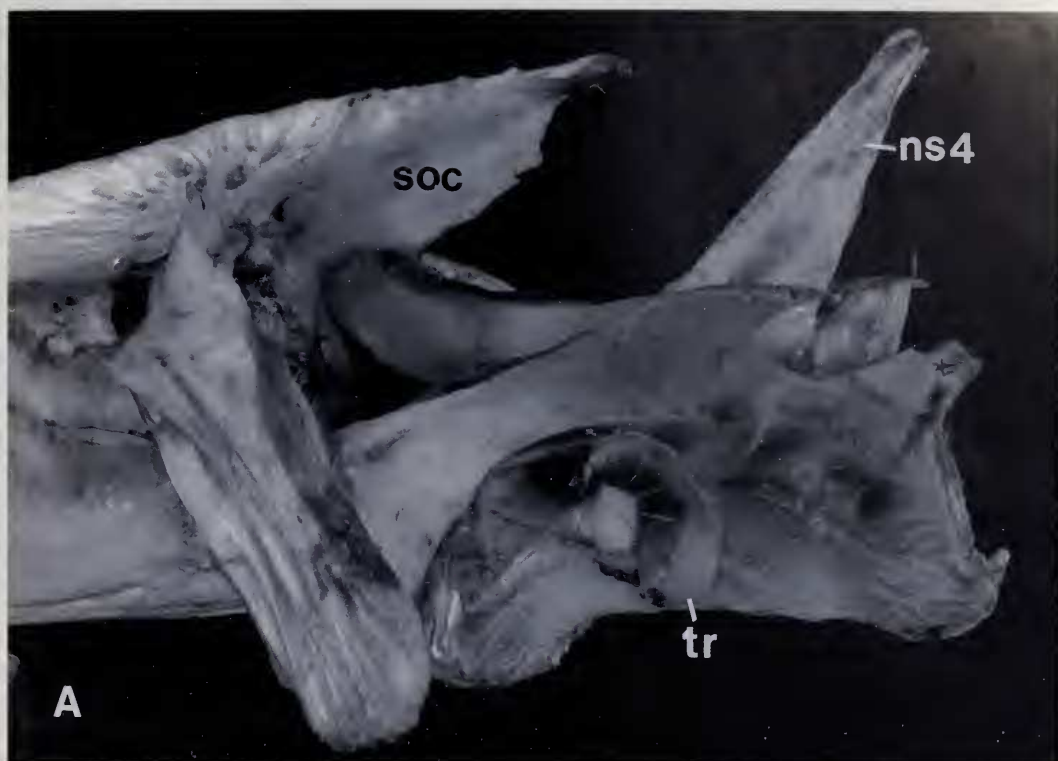
Continuing with Figure 9B, the early stages in the development of the Weberian apparatus/dorsal fin unit were observed. The first two proximal radials of the dorsal fin lengthen ventrally; the first makes contact with neural arch 4 and is positioned between the two halves of this arch. The first dorsal fin spine is complete while the second spine continues to ossify along with its corresponding dorsal fin radial. A median cartilaginous element, called a nuchal plate by some authors (e.g., Teu-gels, 1996), a supraneural by others (e.g., Lundberg, 1982; Lundberg & McDade, 1986; Grande & Lundberg, 1988; Grande & de Pinna, 1998), and a radial or pterygiophore by others (e.g., Brown & Ferraris, 1988; Fink & Fink, 1996), forms anterior to the first dorsal fin radial. This structure, when ossified, articulates with the supraoccipital bone, forming a connection between the dorsal fin and the skull. The homology of this structure is at times perplexing. Lundberg (1982) and Grande and Lundberg (1988) consider this structure to be a serial homologue of the dorsal fin radials, not a nuchal plate, which is involved in the locking mechanism of the second dorsal fin spine; nor do they consider it to be a supraneural. We agree with their assessment. In *I. punctatus* this element forms almost directly dorsal to the supraneurals associated with the Weberian apparatus and in the same plane as the dorsal fin ra-

dials. It therefore does not seem possible for this element also to be a supraneural. Additionally, we agree with Mabee (1988) and Grande and Bemis (1991), who reviewed the homology of radials and supraneurals in centrarchid and polyodontid fishes and concluded that radials are not homologous with supraneurals. Mabee (1988) also showed that the ossification of radials does not always occur in an anterior to posterior direction (e.g., anal fin radials in centrarchids). In *I. punctatus* this anterior element ossifies last. Thus, because of the placement of this element directly dorsal to and in addition to the supraneurals found in *I. punctatus*, and because this element is in direct sequence with the dorsal fin radials, we refer to it as an anterior radial, following Fink and Fink (1996) and reflecting its derivation.

As development of Weberian apparatus/dorsal fin complex continues (Fig. 9C), the supraoccipital crest, which will articulate with the anterior radial, begins to form posteriorly. The claustrum is at least 50% ossified; the horizontal process of the scaphium is rounded anteriorly and will articulate with the back of the skull via the exoccipitals. The first dorsal fin radial is partially ossified and sits between the two halves of neural arch 4. This arch has elongated into a spine that articulates with the first dorsal fin spine and the anterior nuchal plate. The second dorsal fin radial is partially ossified, and its spine consists of two segments bounded by lepidotrichia. All other dorsal fin radials have elongated but remain cartilaginous.

As the developing catfish enters the foraging period, the remaining developmental stages are devoted to further ossifying already formed structures (i.e., making the final attachments among the Weberian ossicles, the dorsal fin skeleton, and the skull) and growth. As seen in fishes of 30 mm TL and larger, the transverse process of vertebra 4 develops two expansions. The anterior part of the transverse process is massive and articulates with the ventral process of the mesial limb of the supraclithrum (Lundberg, 1975). The posterior part of the transverse process (p4p) is thinner, shorter, and positioned horizontally. The posteromedial margin of p4p forms a crescent-like indentation and articulates with the anterior margin of the transverse process of the fifth vertebra (Figs. 11A

FIG. 11. Photographs of skeletons of *I. punctatus* showing in **A** the connection between the anterior part of the Weberian apparatus and the back of the skull (FMNH 73900), and in **B** the fully developed and articulated Weberian



apparatus/dorsal fin unit (FMNH 16711). In B, note the tight connection between neural arch 4 and the first two dorsal fin radials. Anterior is to the left. For abbreviations, see p. 4.

and B). The neural arches of the compound centrum enlarge and fuse with the anterior supra-neural.

As shown in Figure 9D, complex ossifications are associated with the dorsal fin. The anterior and posterior nuchal plates have ossified in fishes of more than 35 mm TL. The anterior radial has ossified fully and has made contact with both the anterior nuchal plate and the supraoccipital. Ossification of the first proximal and distal radials is complete, and the first proximal radial forms a tight connection with the posterior margin of the neural arch complex of the Weberian apparatus. The first dorsal fin spine helps form a locking mechanism for the second fin spine. The second proximal radial is ossified, and its distal tip articulates with the Weberian apparatus. Eventually the entire second radial will abut the first, which in turn is connected with the Weberian apparatus. In fishes of about 40 mm TL, proximal radials 3–6 are ossified (proximal radial 7 and distal radials 3–7 are still cartilaginous). They have elongated and extend between the two halves of neural arches 5–9, preventing them from meeting at the dorsal midline. Instead of the typical midline closure of the arches, as seen with the more posterior ones, an ossified horizontal rod connects the two halves of each arch at least one-third the length of the neural arch. The distal tip of each radial is positioned between the two halves of each arch but above the rod connecting the two halves.

In a fully ossified Weberian apparatus/dorsal fin complex (Figs. 12A and B) a solid bony connection is made between the Weberian apparatus and the dorsal fin skeleton via the spine of neural arch 4 and the anterior dorsal fin radials. It is interesting that the anterior radial articulates with the supraoccipital, forming a connection with the skull (not shown in Figs. 12A and B). When fully ossified, the Weberian apparatus/dorsal fin complex forms at least three connections with the back of the skull. The first one, as just stated, is the articulation of the anterior radial and the supraoccipital crest; the second is by means of an articulation between anterior neural arch 3 and the ventral side of the supraoccipital (Fig. 9D); and the third is via the Weberian ossicles (i.e., scaphium, claustrum, and anterior process of the tripus) with the exoccipitals.

The functional significance of a connection between the Weberian apparatus and the dorsal fin is not certain. Reed (1924) argued that since catfish spines remain in the structural and functional state of a soft ray far into the foraging stage, the

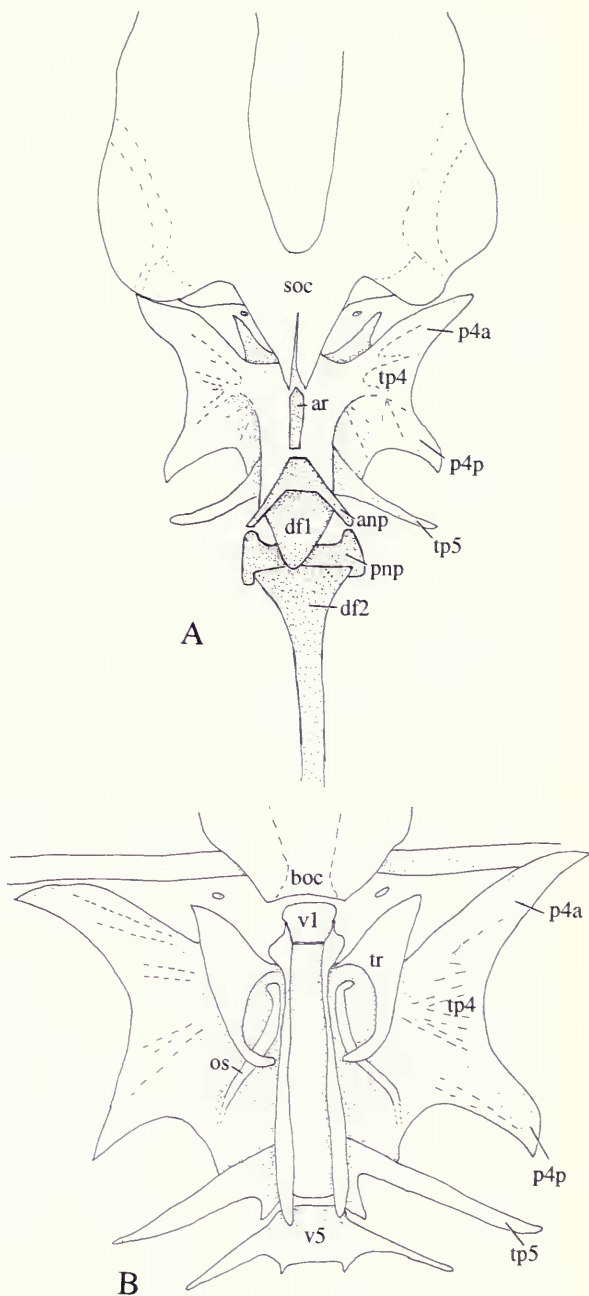


FIG. 12. A. Anterior vertebral column of the specimen figured in Figure 9D, 38.5 mm TL, in dorsal view. B. Illustration of specimen figured in Figure 9D in ventral view. Anterior is directed upward. For abbreviations, see p. 4.

dorsal fin can scarcely be considered a weapon or an element of active defense. Alexander (1965) argued that the principal function of catfish spines is protection, and that the locking mechanism of the dorsal fin spine must be firmly mounted and reinforced by a strong skeleton in order for it to be effective. Essentially, the added skeletal support of the ossified anterior radial and the attachment of the dorsal fin to the skull and Weberian apparatus are aids to stabilize the locking of the second dorsal fin spine. Alexander (1965) and Adriaens (1998) suggested, however, that the locking mechanism of the spine is a pre-adaptation for sound production. Although stridulation by means of pectoral fins has been demonstrated in *I. punctatus* (Fine et al., 1996), the dorsal fin of *I. punctatus* does not possess the necessary opposable serrated pterygiophores commonly associated with sound production. We thus do not consider sound production a possible function for this unit. We do, however, entertain the possibility of sound perception or transmission as a viable, although admittedly inefficient, function for the Weberian apparatus/dorsal fin attachment. The Weberian apparatus transmits high-frequency vibrations from the gasbladder to the inner ear. The possibility of transmitting low-frequency vibrations from the water to the inner ear via this unit is theoretically plausible and intriguing. Physiological experiments are necessary to explore this hypothesis.

Variation in Development

The availability of such a large developmental sample size has afforded us the rare opportunity to examine individual variation within and between series.

The overall pattern of development shown by the 18 stages is very similar for all three series. The rate of development is faster in the earlier stages and then slows in the later stages, about stage 12 for series C and stage 13 for series A and B. The pattern, of course, depends on the stage criteria, which were determined by our selection, but nonetheless accurately reflects the rapid changes occurring in early development of the axial skeleton relative to later development.

The second point is that although the three series follow the same development in sequence as represented by the 18 stages, there is a distinct difference in the age profile of series C compared with series A and B. At every stage up to 16, specimens of series C are older than specimens of

series A and B. In contrast, the ages of specimens of A and B are similar at each stage. This age differential appears to result from the development of the axial skeleton beginning (stage 2) at an earlier age in series A and B than in series C (see Table 1). Although the magnitude of the difference in ages at each consecutive stage fluctuates somewhat, the differential is maintained until stage 16 (from stage 16 to stage 18, age data are not available for all three series). The most likely explanation for this age difference would be incubation and rearing conditions, particularly temperature. However, all series were reared under approximately the same conditions, with temperature about 26°C. Series A was reared in a greenhouse in Chicago, series B at a private hatchery in Missouri, and series C in a Department of Agriculture research unit in Mississippi. The potential for uncontrolled environmental effects is high but cannot account for both the similarity in age profiles between series A and B and the dissimilarity in profiles between series C and series A/B. The origins of the three series offer another possibility. Both series A and B were spawned at a hatchery in Missouri, although several years apart. The hatchery series and the research unit series are possibly separate populations with small inherent differences in the timing of developmental events.

Size as measured by total length also varies among the three series. Again, the differences in size are greater between series C and series A/B than between series A and series B, but the magnitude of the differences is not large, and frequently the size ranges for the three series overlap (see Table 1). In general, series C tends to be slightly larger at each stage than series A or B, possibly the result of being slightly older.

We defined concurrent features as additional variable characters that occur during a stage but that are not restricted to appearing only in that stage in all specimens. Clearly, age and growth in length vary in timing in relation to specific morphological developmental events (stage criteria) and can be classified as concurrent features. In this study, however, size is a slightly better predictor of morphological development than age.

We found a number of other concurrent features that also exhibit individual variation within and between series. Among sensory structures, the pairs of barbels appear in earlier stages in series C than in series A and B, but the appearance of the lateral line marking the beginning of the frontal bone occurs one stage later (stage 6) in series

C than in series A and B. Among other skull elements, the dentary bone forms one stage later (stage 4) and the hyomandibula begins to ossify almost seven stages earlier (stage 5) in series C than in series A and B. In the paired fins, the pectoral girdle and fin are completely ossified at an earlier stage in series C (stage 11) than in series A and B (stage 15). Of the medial fins, the distal radials of the anal fin form at an earlier stage in series C (stage 7) than in series A and B (stage 9). There are several variations in the caudal fin. The parhypural and hypurals 1–5 begin to ossify (stage 6 versus stage 11) and uroneural 1 forms (stage 5 versus stage 8) at earlier stages in series C than in series A and B.

In respect to five structures—the hyomandibula, pectoral girdle/fin, anal fin radials, hypural/parhypural, and uroneural 1—the larvae in series C were smaller than their counterparts in series A and B. In respect to only two structures, the hyomandibula and pectoral girdle/fin, were the larvae of series C younger than their counterparts in series A and B when the concurrent features occurred. There is no obvious pattern to the variation; it does not seem to track age, size, or morphological development as defined by stage criteria. Only in the caudal fin does the variation occur in a consistent manner, which might provide a slightly earlier advantage in escape behavior for yolk sac larvae in series A and B. But much of this variation is probably the result of different structures, or of systems having different developmental rates that are responsive to a variety of factors.

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